STUDIES ON THE IMMUNITY OF THE CALF TO COLIBACILLOSIS

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SUMMARY

The role of colostral whey and immunoglobulins in the aetiology and pathogenesis of colibacillosis in calves was investigated.

Newborn colostrum deprived calves were given colostral whey intraperitoneally prior to challenge with a pathogenic E.coli serotype O78K80(B). Administration of whey above a certain level, calculated in terms of its immunoglobulin content, protected calves against septicaemia but failed to inhibit enteric disease as manifested by severe diarrhoea. In order to prevent colisepticaemia the effective protective dose (ED) 95/30 kg. body weight of the pool of whey used contained ·26g. immunoglobulin M (IgM) and 1·5g. immunoglobulin G (IgG).

Purified colostral IgM and IgG given separately to calves, at levels in excess of those found in the ED.95 of whey, both failed to prevent septicaemia. However, IgM significantly prolonged survival time ($p < 0{\cdot}016$) and delayed the onset of septicaemia ($p < 0{\cdot}014$) when compared with untreated control calves challenged with the same serotype.

In order to investigate the protective function of IgM further, a crude IgM-enriched preparation was isolated from pooled abattoir blood by euglobulin precipitation. This preparation, even in small doses again prolonged survival time ($p < 0{\cdot}{\cdot}002$) and delayed the onset of septicaemia ($p < 0{\cdot}001$) when given intraperitoneally to neonatal agammaglobulinaemic calves which were subsequently challenged with E.coli serotype

O78K80(B). At higher doses, occasionally septicaemia was inhibited completely but this occurred sporadically. Like the whey, IgM similarly failed to influence, to any extent, enteric disease.

To prevent septicaemia consistently it was found necessary to administer the IgM fraction intravenously in two doses given at a four day interval. Serological and immunological examination of these calves' sera revealed that the levels of passively acquired IgM dropped rapidly within a few days and this drop was paralleled by a similar fall in specific antibody to the challenge E.coli serotype O78K80(B) as measured by indirect haemagglutination and antiglobulin tests.

From these results, it was concluded that IgM was the immunoglobulin responsible for the protection of the calf against septicaemia. Moreover, since colostrum, under natural conditions protects the calf from septicaemia and enteric disease, it was postulated that colostral immunity was of a complex nature involving two separate systems (1) systemic - preventing septicaemia and (2) local - within the lumen of the small intestine, inhibiting enteric disease.

The local intestinal protective function of colostral whey was demonstrated in hypogammaglobulinaemic calves. Market calves, under one week old were divided into four groups, (1) control calves, (2) calves which were given an IgM rich fraction intravenously, (3) calves which were given an IgM

rich fraction intravenously and colostral whey orally and (4) calves which were only given colostral whey orally. They were placed collectively in premises previously contaminated by calves with colibacillosis. All the control calves died, 5 with colisepticaemia, 2 with severe diarrhoea. The IgM fraction administered alone inhibited septicaemia in all cases, but not enteric disease. Calves given the IgM fraction and colostral whey orally had prolonged survival times ($p < 0.02$) over the other three groups and the onset of diarrhoea was significantly delayed ($p < 0.007$). Since in this study it was demonstrated that the colostral whey had not been absorbed from the small intestine, it is concluded that colostral whey in addition to providing systemic immunity has a local protective function within the gasgro-intestinal tract.

From the proximal small intestine of calves which had severe diarrhoea, mucoid strains of E.coli were isolated in large numbers. Several of these isolates were shown to belong to enterotoxigenic strains as adjudged by their ability to dilate ligated rabbit intestinal loops. One of these serotypes O101K(A?) was administered orally to colostrum-deprived calves which had been protected systemically from septicaemia by the intravenous use of the IgM preparation. 13 calves so treated suffered from severe diarrhoea within 24 hours of infection and 11 died. None of the calves was bacteraemic but the challenge organism was isolated from the proximal small intestine of all those which died and from the faeces of the 2 survivors.

It was considered that this was an acute form of enteric colibacillosis. In the calves which died, there was very marked haemoconcentration, the packed cell volume increasing by as much as 50% of pre-challenge levels. It was postulated that the haemoconcentration may have resulted from the absorption from the small intestine of endotoxin. Immunological analysis of daily faeces samples demonstrated the presence of immunoglobulins of the IgG, IgM and IgA classes within 48 hours of infection.

From the results of the complete study, it is concluded that colostral immunity to colibacillosis is of a complex nature involving two separate, independent systems, (1) systemic - mediated largely by IgM - preventing septicaemia and (2) locally within the gastrointestinal tract inhibiting diarrhoea. Moreover, for the survival of a calf in a contaminated environment, it is necessary that both systemic and local intestinal protection be present in adequate proportions.

CHAPTER I.

GENERAL INTRODUCTION.

History and Incidence.

Neonatal diarrhoea is not a modern disease; indeed, under a variety of synonyms such as "scours", "white scours" and "calf scour" it has been recognised as an important problem for at least 200 years. Tolnay (1799) was the first to record the condition in young calves and he and White (1825) attributed the disease to indigestion, resulting from improper feeding. Later, various authors (Obich, 1865; Frank, 1876; Dieckerhoff, 1891) suggested that infectious agents were implicated in the aetiology and this was further supported by Jensen (1893, 1897) who described the successful reproduction of the condition in newborn calves, from the organs and blood of which he isolated Bacterium coli - now known as Escherichia coli (Nomenclature Committee of the International Association of Microbiologists Enterobacteriaceae Subcommittee report, 1954). Since that time numerous workers (Poels, 1899; Smith & Little, 1922; Smith & Orcutt, 1925; Mejlbo, 1934; Lovell, 1937) have stressed the association between E.coli and gastro-intestinal disease so that today, the syndrome is referred to as colibacillosis.

Colibacillosis has a world wide distribution and is present in all cattle raising countries. In Britain, over the past 40 years, numerous surveys have been carried out in an attempt to ascertain the extent and causes of death among young calves. Many of these are of limited value, because

they have involved only relatively small populations of cattle, often within a limited area and are thus not representative of the whole country. In 1933, Jordan noted, that in Ayrshire, there was a 20% mortality of heifer calves. Lovell & Hill (1940) reported, that 5·5% of female calves in England and Wales and 11·9% in Scotland died, before they were 6 months old. Withers (1952 & 1953) found an overall mortality of 8·3% and again observed considerable regional variation, losses again being higher in Scotland. Sellers, Smith & Wood (1968) quoted a mortality of 4·9% whilst the most comprehensive study to date (Leech, Macrae & Menzies, 1968) recorded that during 1962, 4% of all calves born, died under one month old.

Abroad, surveys have revealed similar figures. In Ireland, Murphy (1955) reported that 17·1% of calves in West Limerick died, whilst Gracey (1960) carried out a survey on 1 in every 50 farms in Northern Ireland and found a 2·8% loss. In the United States of America, Dollahite, (1939 a & b), investigating the mortality in a large herd found, that, during the first 6 months of 1938, 49% of calves died before they were 5 days old and Erd, Gilden, Goodman, Millard & Murdock (1951) reported a 24% mortality in young calves housed in conventional covered sheds. In Denmark, the death rate during the 1950's varied between 7 and 10% (Ottosen, 1959) and Van Dieten (1963), in nearby Holland, quoted losses of 6·1%.

These surveys also revealed that there was considerable

seasonal variation, mortality being highest in late winter and early spring (Jordan, 1933; Gracey, 1960; Murphy, 1955) and that there was a higher incidence in dairy herds, than in beef herds (Withers, 1952; Leech, Macrae & Menzies, 1968). It is now known that these differences relate directly to the difference in the management of beef and dairy herds (McBeath, Penhale & Logan, 1971). Whilst the overall death rate was 4 - 8% it should be realised that losses were often confined to problem herds where mortality could be higher than 20%. (Jordan, 1933; Smith, 1934; Ottosen, 1959; Dam, 1960).

Post mortem examination of calves under one month old, has shown that colibacillosis was the commonest cause of death. Jordan (1933) considered that the majority of losses (92%) were due to scouring and navel infections. Lovell & Hughes (1935) examined bacteriologically 100 cadavers, from a wide area of Britain and concluded that at least 37% of deaths were due to E.coli infections. Withers (1952) stated that in his survey 26·9% of deaths were due to E.coli. Of 3,667 calves examined during 1959 - 1961 by the Veterinary Investigation Service of the Ministry of Agriculture, Fisheries & Food (Hughes, 1964) it was found that the commonest syndromes were colisepticaemia (24·5%) and gastro-enteritis (29·4%). A later examination of 350 carcases, in the year 1962 - 63, (Leech, Macrae & Menzies, 1968) revealed a 25% incidence of enteritis. In Northern Ireland, Gracey (1960) was of the opinion that the commonest causes of death were

colisepticaemia and white scours which accounted for 24% of losses, whilst in Denmark, the State Veterinary Serum Laboratories reported that colisepticaemia and gastro-enteritis accounted for 19·5% and 42·8% of deaths respectively (Ottosen, 1959).

From the above surveys, it can be seen that there has been a gradual drop in calf mortality over the past 20 years. Nevertheless, it is still a grave economic problem. In one year, Leech, Macrae & Menzies (1968) indicated that 108,000 calves under one month old died in Great Britain and that gastro-enteritis was responsible for nearly half (44·5%) of these deaths and in the farming press (Farmers Weekly, 1971) the figure of 182,000 annual deaths has been quoted. In the United States of America, Amstutz (1965) stated that calf losses annually accounted for 40 million dollars. The above figures relate only to dead calves and do not take into consideration the higher morbidity of the disease (Leech, Macrae & Menzies, 1968) nor the unthriftiness of many of the recovered animals (B.V.A., Handbook).

Aetiology and Pathogenesis of Colibacillosis.

Under natural conditions, it has been quoted (Gay, 1965) that colibacillosis may exist in two main forms. (1) A septicaemic form in which E.coli invade the circulation and organs and (2) an intestinal form - characterised by diarrhoea - in which E.coli are generally confined to the small intestine and do not invade the circulation, except occasionally, during

the terminal phase of illness.

The septicaemic form is frequently seen in calves which have been deprived of colostrum (Smith & Orcutt, 1925; Smith, 1962; Fey & Margadant, 1961). It is usually acute and often causes death within 48 - 72 hours of birth. Clinically, it is characterised by fever, loss of muscular tone, followed by prostration and death. Diarrhoea may, or may not be a feature and in more chronic cases, omphalophlebitis and polyarthritis are often noticable. Mainly as a result of transmission experiments, (Dunne, Glantz, Hokanson & Bortree, 1956; Fey & Margadant, 1961; Penhale, 1965) it is now generally accepted that E.coli can act as a primary aetiological agent in this syndrome.

Penhale (1965) demonstrated that the accumulation of endotoxin in the blood and organs was responsible for the pathogenesis of this form of the disease. It is thought that endotoxin causes an anaphylactoid reaction, with the liberation of histamine and other substances which cause circulatory failure (Braude, 1964). In the calf, blood is pooled in the lungs, probably as a result of constriction of pulmonary veins and in severe cases oedema develops (Penhale, 1965). As a sequence to the oedema, haemoconcentration develops. Hypoxia also occurs, causing loss of capillary tone and increased permeability which contributes to further vascular collapse. It has been found that a comparatively small number of serotypes of E.coli has

been isolated from naturally occurring cases of septicaemia (Bokhari & Ørskov, 1952; Ulbrich, 1954; Fey, 1957) and of these, serotype O78K80(B) appeared to predominate. Other authors (Rees, 1958; Dam, 1962; Sojka, 1965) have confirmed these results and it appears that this serotype has a special pathogenicity for the calf. Why certain strains are more pathogenic in this syndrome than others is not known, but Smith (1962) has postulated, that it is connected with their ability to survive in precolostral calf serum. Precolostral serum, though devoid of antibodies, is bacteriocidal for several E.coli serotypes (McEwen, 1950; Glantz, Dunne, Heist & Hokanson, 1959; Smith, 1962; Penhale, 1965). This action, which is thought to be non-specific, is due to the presence of a heat labile factor called properdin, which has the ability to fix complement (Pillemer, 1956).

The intestinal syndrome which is more chronic than the septicaemic form is characterised by a profuse white diarrhoea which often has a very distinctive smell. As a result of the diarrhoea, which can exceed two litres daily, there is dehydration, loss of weight, weakness and a marked alteration in blood chemistry. Whilst plasma potassium and urea levels rise, sodium and bicarbonate ions are lost, causing a resultant fall in blood pH and acidosis (Dalton, Fisher & McIntyre, 1965; Watt, 1965; Fayet, 1968 a & b). As yet, the exact cause of death is unknown. It has been suggested tentatively that the acidosis, accompanied by hyperkalaemia may cause cardiac

arrhythmias and terminally heart block (Fisher, 1965; Fisher & McEwan, 1967). In addition to the chronic form of diarrhoea, an acute syndrome has been described (Gay, McKay & Barnum, 1964b) in which calves are suddenly affected with a very profuse watery diarrhoea, accompanied by marked haemoconcentration, followed by death within 24 - 36 hours. Little is known of this condition which has been called enteric-toxaemia, on the grounds that endotoxin may have been absorbed from the small intestine. Whilst early workers stressed the association of E.coli with gastro-enteritis, the significance of E.coli in the aetiology of this condition is still a matter of some controversy. This has arisen primarily because of the equivocal results of many experimental transmission experiments. Smith & Little (1927), McEwen (1950), Gay, McKay & Barnum (1964c) and Smith (1962), all failed to produce the condition with isolates from natural occurring cases and Smith was of the opinion that E.coli was only of secondary significance. In addition to E.coli, other aetiological agents have been incriminated; Baker (1943) isolated a virus from scouring calves; Blaxter & Wood (1953) considered that an upper intestinal tract failure was responsible and Bywater & Penhale (1969) suggested that a lactase deficiency may be associated with the pathogenesis of the condition.

With the serological classification of Entero-bacteriaceae by Kauffmann (1954), it has been possible to

examine, in greater depth, the organisms isolated from the intestine of scouring calves. Authors were able to correlate the appearance of outbreaks of scour with the presence of a particular serotype of E.coli. The position was further clarified when Taylor, Malby & Payne (1958), adapting a technique first used by De & Chatterji (1953), demonstrated that in rabbit intestinal loops, certain mucoid strains of E.coli could cause dilation accompanied by a purulent inflammation. Later, Taylor, Wilkins & Payne (1961) confirmed the observations of De, Bhattacharya & Sarkar (1956) that there was a definite correlation between strains causing dilation and those associated with diarrhoea in infants. Calf strains did not give quite the same degree of correlation, but as the authors observed, some of these strains, apart from being stored for several years, may have been isolated from septicaemic calves and, as we now know, septicaemic strains do not dilate intestinal loops (Smith & Halls, 1967a). Smith & Halls (1967 a & b) developed the technique still further, in domestic animals, and found that enteropathogenic strains showed considerable host specificity. Furthermore, they demonstrated that these strains produced an enterotoxin - an observation previously recorded with regard to Vibrio cholerae (De, Ghose & Sen, 1960) - which was responsible for the dilating effect and that the anterior small intestine was more susceptible than the distal portion. It is now known that enterotoxin exists in two forms (Smith & Gyles, 1970)

which, in spite of certain physical differences, have the same biological activity. Moreover, it has been shown (Smith & Halls, 1968a) that enterotoxin production is controlled by a genetic factor known as a plasmid and that this factor is transmissible to other negative strains of E.coli, conferring on them the ability to produce enterotoxin.

Using a Thiry-Vella loop system in calves, Bywater (1971) was able to demonstrate that, in vivo, enterotoxin caused a significant shift towards secretion into the lumen of the small intestine of fluid, sodium, chloride and bicarbonate ions. Endotoxin on the other hand had little or no effect.

As yet, it is not known how enterotoxin causes dilation, in vitro, or diarrhoea, in vivo, and in the latter context, research has been badly hampered by an inability consistently to reproduce the syndrome experimentally. Histologically, Ingram (1958) has suggested that there is little evidence of microscopic change in the small intestine of scouring calves. More detailed work in pigs, (Kohler, 1967a; Kenworthy, 1970; Bohl & Cross, 1971) tends to support Ingram's observations. In this species, it has been shown that to produce diarrhoea organisms do not need to penetrate the lumen or epithelial cells of the small intestine (Drees & Waxler, 1970 a & b) and this is in keeping with observations that sterile cultures or isolated enterotoxin are capable of producing diarrhoea (Smith & Halls, 1967b; Kohler, 1967b). Furthermore, Bywater (1971) was unable to demonstrate absorption of

enterotoxin in his experiments.

In the past, it has been considered that, in addition to E.coli, other aetiological factors may be involved. These factors were nutritional, environmental, or related to host resistance.

Host Resistance.

From the numerous surveys, it is clear that colibacillosis is a disease only of neonates and that age confers resistance upon the calf. This has also been supported experimentally. (Smith & Little, 1927; McEwen, 1950; Smith & Halls, 1967a). As the calf is immunologically competent at birth, (Brown, 1956; Fennestad & Borg Petersen, 1957; Penhale, 1965; Klaus, Bennett & Jones, 1969), it is probable that this resistance is due to the presence of antibodies. The incidence of death is highest in the first fortnight of life; affected calves surviving this period, often recover (Roy 1970). Blakemore, Davies, Eylenburg, Moore, Sellers, Worden (1948) and Withers (1952) suggested that Ayrshire and Channel Island breeds were more susceptible than Friesians but no breed difference was observed by Aschaffenburg, Bartlett, Kon, Roy, Sears, Ingram, Lovell & Wood (1952) who compared Friesian and Shorthorn bull calves.

Environmental Factors.

As previously stated, there is a marked seasonal incidence in disease. Jordan (1933), Lovell & Hill (1940), Withers (1952) and Gracey (1960) reported that the highest mortality

occurred during late winter and early spring and it was thought that this may be associated with low temperatures and absence of sunshine (Withers, 1952), but McEwen (1950) could find nothing to support this, nor did Roy, Palmer, Shillam, Ingram & Wood (1955) who reared Shorthorn calves out of doors during winter. It is more likely that the high mortality, in the late winter and spring, is due to the fact that, when calf pens are continually in use, there is a build up of "infection". Successive calves become more difficult to rear and in the environment there is a dominance of virulent strains of E.coli (Roy et al, 1955; Wood, 1955). Withers (1952) was of the opinion that individual treatment of each calf was of prime importance in preventing scour; a view also supported by Gracey (1960), who believed that the low incidence in Northern Ireland, was due to small farm units, where often the farmer's wife took special care of calves. Fisher, Selman, McEwan and de la Fuente (1968) are convinced that the seasonal incidence is merely a reflection of the difference in management, particularly in dairy cows, between summer and winter. In winter, cows usually calve in byres and the calves are immediately removed, thus depriving them of the chance of obtaining colostrum. By calving cows in individual boxes and allowing the calves to remain with their dams - a situation similar to that of cows calving on grass in summer - Fisher et al (1968) eliminated the effects of winter practice. The lower incidence reported in beef suckler herds strongly supports

this hypothesis.

Nutritional Factors.

Several workers have emphasised the importance of nutrition of both the dam and the calf as predisposing factors. In relation to the dam, Payne (1949) reported that the incidence of scours was less frequent when cows were fed large amounts of green silage or dried grass, than when a high concentrate diet was fed. In this, he was supported by Inglis (1960), but paradoxically, Shanks (1950) reported the occurrence of a toxic substance in clover rich grass which could be passed in the milk to calves, causing a persistent diarrhoea. In respect of the calf, because of the importance of Vit. A in the maintenance of the integrity of epithelial cells it has been postulated that a deficiency of this vitamin may render the calf more susceptible to scour (Stewart & McCallum, 1938) but vitamin A supplements have been unsuccessful when used therapeutically in calf diarrhoea (Greig 1943; Aschaffenburg, Bartlett, Kon, Roy, Sears, Thompson, Ingram, Lovell & Wood, 1953; Blakemore et al, 1948). That overfeeding with milk can initiate scour has been suggested by Pounden & Hibbs (1947). Sheehy (1934) and Roy (1970) have stated that sudden changes in diet, the use of sour milk and poor hygiene may all play their part; but McEwen (1950) subjected calves to extreme variations in feeding, by giving feeds irregular in timing, volume and temperature and concluded that, provided a calf had been fed colostrum, he could find no evidence to support

that these variations adversely influenced the calf's performance. More recent experiments (Mylrea, 1966; Roy, Shillam, Thompson, Stobo & Greatorex, 1964) support McEwen's findings. Fisher et al (1968) also attempted to "overfeed" calves by allowing them to drink to satiation and found that calves only took approximately four pints of milk, when suckled, or two pints, when bucket fed.

It is now clear that the chief nutritional factor affecting the calf's ability to withstand neonatal infection is whether or not it obtains an adequate intake of colostrum during the first 24 hours after birth. That colostrum feeding reduced mortality was first recorded by Jensen (1893), who reported that if calves were fed boiled milk on the first day postpartum, most of them died from acute diarrhoea but if fed colostrum on the first day, they survived. These findings were subsequently confirmed by others (Joest, 1903; Titze & Weichel, 1908; Smith & Little, 1922) and it is now accepted that colostrum in adequate quantities, confers immunity on the neonatal animal.

Colostral Immunity.

Ehrlich (1892) first demonstrated that mice, rendered immune to toxic substances, could transfer this immunity to their young through the placenta and colostrum, but it was not until the phenomenon was examined in detail in the calf, by Smith and his co-workers in a series of papers between 1922 and 1930, that the nature of this immunity was clarified.

(Howe, 1921; 1922; Orcutt & Howe, 1922; Smith & Little, 1922; Smith, 1930; Lyttle & Orcutt, 1922). These authors showed that the sera of newborn calves did not contain agglutinins or antibodies until they had ingested colostrum. Initially, they looked for Brucella abortus agglutinins using colostrum from vaccinated cows, but later recorded the presence of antibodies to scour producing strains of E.coli in the colostrum of normal cows. They defined the changes occurring in the serum proteins of the newborn calf following the ingestion of colostrum and demonstrated, that the globulin fraction was absorbed without change in sufficiently large amounts to alter substantially the composition of the blood. Very significantly, they observed that this type of absorption was limited to the first 24 - 36 hours after birth. Later studies, using electrophoretic and ultracentrifugal techniques, have confirmed that there is no detectable change in the colostral globulin or immune lactoglobulin, as it was now called, during absorption into the serum of the newborn animal (Jameson, Alvarez-Tostado & Sortor, 1942; Johnston & Pierce, 1959). In 1949, Aschaffenburg and his co-workers fed calves various isolated fractions of colostrum and confirmed that the prophylactic action of colostrum was in the whey fraction and that only calves which had received either whey, which contained the immune lactoglobulin, or the lactoglobulins themselves, survived and thrived normally.

Immune lactoglobulins are absorbed from the small

intestine (Comline, Roberts & Titchen 1951 a) and are carried in the lymph to the peripheral blood, little appearing in the portal vascular system. The globulins are absorbed, by micropinocytosis, into the columnar cells of the epithelium and transferred across them in vacuoles (Comline, Roberts & Titchen, 1951b; Clark, 1959). This is a very rapid process and gammaglobulin can be detected in the thoracic duct lymph, within 80 - 120 minutes of it being introduced into the duodenum and 12 - 25% of the protein is recoverable from the lymph, within 300 minutes (Balfour & Comline, 1962). The same authors (1959 a - b) also confirmed, that absorption only occurs during the first 24 hours of life and in their opinion, is not specific, other proteins such as bovine serum globulins, serum albumin and even higher molecular weight polysaccarides being absorbed during this time. Pierce (1960) also found that β lactoglobulin was absorbed but, by virtue of its low molecular weight, was quickly cleared from the calf's blood by glomerular filtration. Nevertheless, it would appear that some specificity does exist, for it has been shown that in other species, homologous lactoglobulin is absorbed preferentially when fed in combination with heterologous lactoglobulin (Halliday, 1955; Pierce & Smith, 1967), and in addition, since most of the data accumulated has been of a qualitative nature, it is unwise to interpret it quantitatively. Balfour & Comline (1962) also found that the absorption of the immune lactoglobulins

was aided by the presence, in the colostrum, of certain catalytic factors which they identified as proteins of small molecular weight, organic and inorganic phosphate compounds. Selman, McEwan & Fisher (1971) demonstrated that the presence of the dam, in some unknown way, facilitated absorption of immunoglobulin.

It has been suggested that a defect in the absorption mechanism occurs quite commonly in 10 - 16% of calves. Fey (1962), Gay (1965) and Penhale (1965) recorded instances, where calves, thought to be colostrum fed, were subsequently found to be agammaglobulinaemic. However, precise information regarding the amounts of colostrum fed and time of feeding were not given and Selman (Selman, McEwan & Fisher, 1971) could find no cases of malabsorption in 50 calves which he had personally fed and casts doubt on previous findings. In this, he is supported by Kruse (1969).

It is apparent that immune lactoglobulins, since they are absorbed intact, must escape proteolytic digestion in the abomasum. Laskowski & Laskowski (1951) suggested, that in colostrum, there is a trypsin inhibitor whilst Fey (1971) stated that, because of the high pH in the abomasum during the first 20 hours of life, there was no peptic activity. As previously stated, several authors have noted that absorption of immune lactoglobulins stops after 24 - 36 hours. From the experiments of Selman, McEwan & Fisher (1970), it would appear that the limits are much narrower and may be as

little as 8 hours. Kruse (1970b) in a more detailed study revealed that, within 6 hours post-partum, absorption was diminished and that, by delaying feeding from 2 to 20 hours, the absorption co-efficient was reduced linearly by 50%. He also observed that efficiency of absorption was not influenced by the percentage of immunoglobulin in the colostrum or by the quantity of immunoglobulin fed to the calf.

It was first suggested by Smith & Little (1922) that colostral antibodies are derived from the maternal serum and subsequent work has confirmed this (Polson, 1952; Pierce, 1955; Askonas, Campbell, Humphrey & Work, 1954; Larson & Gillespie, 1957; Larson, 1958; Feldman, 1961) and it is now known that the udder selectively concentrates the immune globulins of the serum (Garner & Crawley, 1958; Pierce & Feinstein, 1965).

That antibodies were associated with immunity in blood serum had first been recognised by Von Behring & Kitasato (1890). Tiselius (1937) used electrophoresis to demonstrate that rabbit antibodies to egg albumin migrated mainly with the gammaglobulin fraction. In 1939, Tiselius and Kabat absorbed hyperimmune rabbit serum with specific antigen and showed that the electrophoretic profile of the gammaglobulin fraction was significantly altered. With the use of more sophisticated techniques it became clear that antibodies exhibited considerable heterogeneity and that they could not be classified solely according to their chemical, physical or biological properties, because, molecules with similar

chemical or physical properties, had different biological ones and vice versa. For example, it was common practice to classify an antibody according to its functional capabilities as an agglutinin, precipitating or neutralising antibody, without realising that each of these activities could be shared to a greater or lesser extent by the same molecule. In order to simplify the confused terminology then in vogue, the World Health Organisation (1964) adopted the suggestion of (Heremans, 1960) that these antibodies should be called "immunoglobulins" and now they have been defined as proteins of animal origin with known antibody activity as well as other chemically related normal and pathological proteins.

Immunoglobulins which form the humoral immune response, are manufactured by cells of the reticulo-endothelial system. In response to stimulation by antigen, small lymphocytes which are derived from stem cells of the bone marrow, are thought to differentiate into plasma cells which actively produce immunoglobulin. Each plasma cell makes only one class of immunoglobulin and it is probable that individual classes of immunoglobulin found during an immune response are made by different groups or clones of cells (Burnet, 1964). Plasma cells though principally found in lymph nodes and the spleen, from which immunoglobulin is secreted into the blood, are found also in such tissues as exocrine glands, udder, intestinal, respiratory and uro-genital tracts where they manufacture immunoglobulin locally (Crabbé, Carbonara & Heremans, 1965;

Porter, Noakes & Allen, 1970b; Yurchak, Butler & Tomasi, 1971).

The chief characteristic of an immunoglobulin molecule is its ability to combine specifically with the antigen which has stimulated its production.

Structure and Function of Immunoglobulins.

Much of our present knowledge of the structure of immunoglobulin is the result of Edelman's and Nisonoff's work in U.S.A. which was paralleled in England by Porter. All immunoglobulins appear to be either monomers, dimers or polymers of a molecule of 4 polypeptide chains which are made up of amino acid sequences (Fig.I). There are two light chains (L chains - 20,000 molecular weight) and two heavy chains (H chains - 55,000 molecular weight) linked by disulphide bonds and other so called non-covalent interactions such as electrostatic forces and hydrophobic bonds (Nisonoff, 1971). In addition, there are also intra-chain disulphide bonds. Each chain is made up of a region that is invariable within that class of immunoglobulin and a variable portion at the antibody sites. It is this variability which is related to the multiplicity of antibody specificities which can exist in any one class of immunoglobulin. The light chains exist as either Kappa or Lambda chains and are common to all immunoglobulin classes but the H chains are specific to each class, IgG possessing γ , IgM possessing μ , IgA α , IgD δ , and IgE ϵ .

Immunoglobulin structure has been studied by fragmentation of the molecule either by chemicals (Edelman, 1959) or by

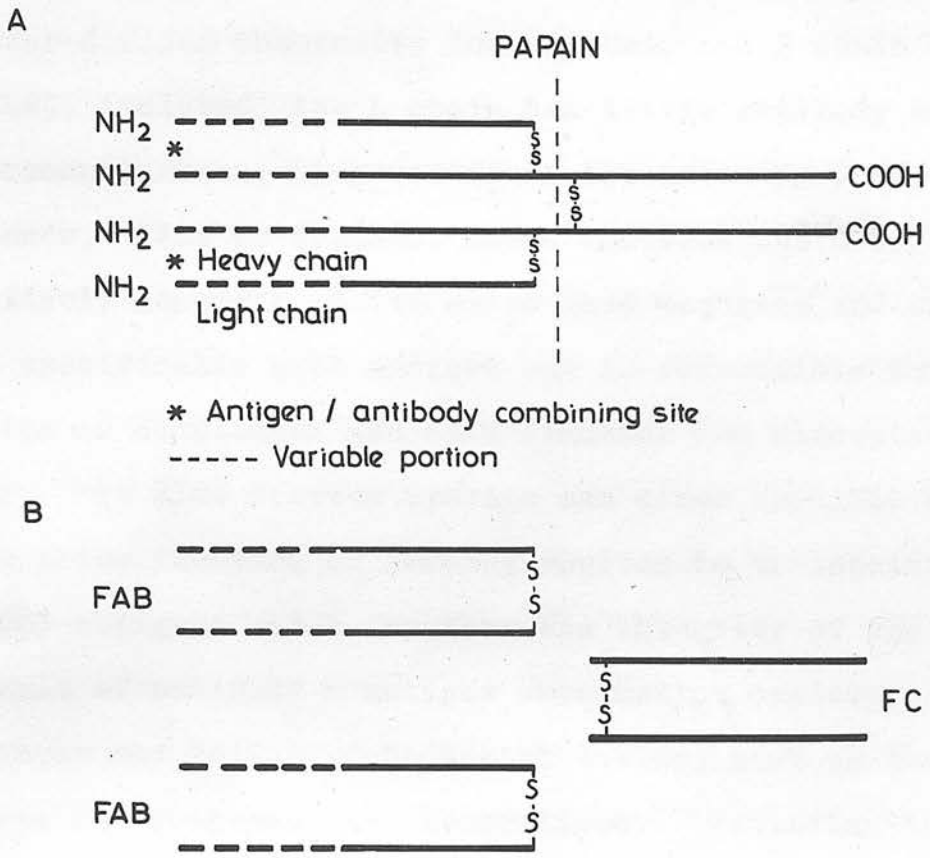

FIGURE I.

A Diagrammatic representation of the structural arrangement of polypeptide chains of an immunoglobulin molecule; intra-chain disulphide bonds are not shown.

B Portions of molecule resulting from splitting by papain.

proteolytic enzymes (Nisonoff, Wissler, Lipman & Woernley, 1960; Porter, 1957 & 1958).

Cleavage with papain yields three fragments; 2 Fab fragments and a single Fc fragment (Fig.1). The Fab fragment contains the NH_2 terminal half of one of the H chains, plus one complete L chain. Together, the NH_2 terminals of these chains contain the antibody combining site. If the Fab portion is further divided chemically into L chain and H chain it is found that, isolated, the L chain has little antibody activity, but on recombination, it potentiates the antibody activity of the H chain. The Fc fragment which contains the COOH terminal is relatively constant in its amino acid sequence and cannot combine specifically with antigen but is responsible for such properties as complement and skin fixation and placental transfer. It also carries species and class specific determinants.

The prime function of immunoglobulins is to inactivate or destroy antigens which threaten the integrity of the host. As a result of antibody - antigen combination various consequences may follow. Bacterial toxins, such as those of tetanus and gangrene, are neutralised. Bacteria, in particular gram negative ones, can be lysed - with resultant death of the organism - in the presence of complement or, they may be agglutinated, which aids phagocytosis. Also immunoglobulins are capable of opsonisation, i.e., the labelling or "gift wrapping" of the antigen to make it more attractive to phagocytes, or precipitation of antigen which again aids its removal by the reticulo-endothelial system.

In man, on the basis of their antigenic determinants, five principal immunoglobulins have been isolated and named immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), immunoglobulin D (IgD) and immunoglobulin E (IgE). Some of these can be further divided into sub-classes, e.g., in the case of IgG there are four; IgG1, G2, G3, G4. In addition to class differences, there are xenogeneic and allogeneic differences within classes of immunoglobulin.

Bovine Immunoglobulins.

In the bovine, three classes of immunoglobulin have been identified, IgG, IgM and IgA. The recent work of Campbell (1970) on milk allergy in cattle, may lead to the identification of IgE. Immunoglobulins are present in blood and most body secretions, but their relative concentrations vary considerably according to the fluid examined. Quantitatively IgG and IgM are the principal immunoglobulins of plasma, whereas IgA is the major one in external secretions, apart from colostrum where IgG predominates (Penhale & Christie, 1969; Klaus, Bennett & Jones, 1969; Porter & Noakes, 1970; Mach & Pahud, 1971). All three are selectively concentrated by the udder and absorbed by the newborn calf (Murphy, Aalund, Osebold & Carroll, 1964; Pierce & Feinstein, 1965). As previously stated, it was considered that absorption of colostrum was unselective and recently Klaus, Bennett & Jones (1969) considered that individually IgG and IgM were absorbed with equal efficiency. In an experiment just completed, (Penhale, Logan, Selman,

McEwan & Fisher, in preparation), individual calves were fed a fixed quantity of a colostrum pool at different intervals post-partum and it was found that there was considerable variation in the absorption of each class of immunoglobulin. IgG was absorbed for a longer period post-partum than IgM, whilst on the other hand, IgA was absorbed for a very much shorter period than the others.

Immunoglobulin G.

This immunoglobulin exists as a monomer and thus has a structure similar to that shown in Fig.1. It is present in most body fluids, being distributed equally between the serum and the extravascular fluids (McDougall & Mulligan, 1969). Quantitatively, it accounts for 85 - 90% of the immunoglobulin in serum and colostrum (Klaus, Bennett & Jones, 1969). On ultra-centrifugation it has a sedimentation co-efficient of 7S (Nolan & Smith, 1962). Using various methods of separation, IgG can be divided into two sub-classes, IgG1 and IgG2. Whilst IgG1 and IgG2 are found in serum in similar amounts, IgG1 is preferentially concentrated by the udder in colostrum (Murphy, Aalund, Osebold & Carroll, 1964; Pierce & Feinstein, 1965). IgG antibodies are very effective at neutralising toxins, lysozymes and viruses (Weir, 1970) but not nearly so efficient at lysing or agglutinating bacteria or red cells in the presence of complement (Humphrey & Dourmashkin, 1969).

Immunoglobulin M.

IgM has a polymeric, stellate configuration, being comprised

of 5 immunoglobulin molecules which are linked by disulphide bonds in the Fc portion (Fig.2). It has a sedimentation coefficient of 19S. On Sephadex G 200, it is eluted in the exclusion peak, whereas IgG is retained. Treatment with 2 - mercaptoethanol dissociates the molecule with resultant loss of biological activity. IgM comprises less than 10% of the immunoglobulin in serum and colostrum (Klaus, Bennett & Jones, 1969; Penhale & Christie, 1969). Unlike IgG, it is essentially an intravascular immunoglobulin, 90% being found in the plasma (Cohen & Freeman, 1960). In the presence of complement, IgM lyses red cells. It has been estimated that a single IgM molecule attached to a red cell by its multiple antibody/antigen combining sites can cause lysis whereas 1,000 IgG molecules are required for the same effect. (Humphrey & Dourashkin, 1969). Similarly, because of its multiple combining sites IgM is a particularly efficient agglutinating antibody. In the rabbit, antibacterial IgM antibody is known to be more than twenty times as active as IgG antibody (Weir, 1970).

Immunoglobulin A.

IgA was first identified in human serum by Heremans, Heremans & Schultze (1959) and in 1961, Hanson described a form of IgA occurring in human milk which appeared to be different antigenically from serum IgA. Later, this form of IgA, now known as secretory IgA, was demonstrated in other

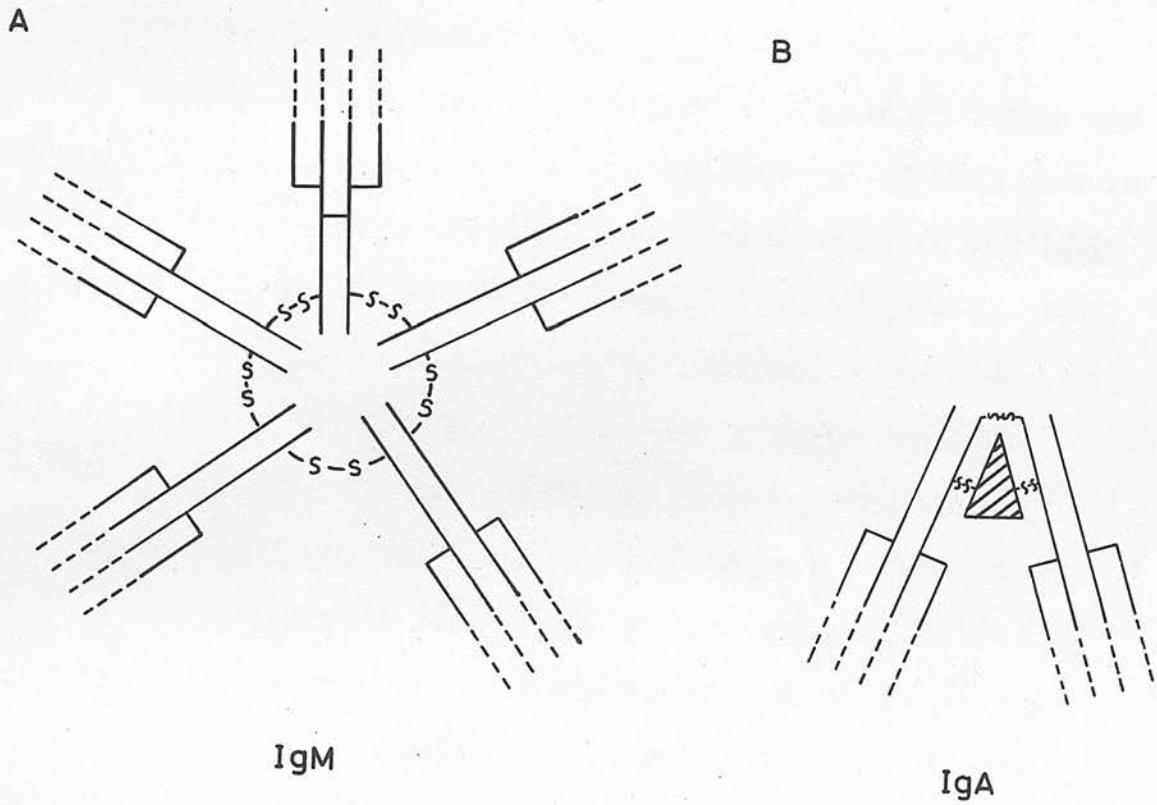

FIGURE 2.

Diagrammatic representation of Immunoglobulin M & A molecules.
A; IgM B; IgA shaded area represents secretory piece.

secretions and it was found to be the predominant class of immunoglobulin there (Tomasi & Zigelbaum, 1963; Chodirker & Tomasi, 1963). It was not until 1969 that IgA was isolated in the bovine (Mach, Pahud & Isliker, 1969). IgA usually exists as a dimer with a sedimentation co-efficient of 9S and it is in this form that it is found in serum. However, in secretions the majority of IgA has attached to it a "secretory piece" (Fig.2) and has a sedimentation co-efficient of 11S. This secretory piece (Tomasi, 1967) which is attached to the IgA by covalent disulphide bonds, has no antibody activity. Various functions have been attributed to secretory piece and currently it is thought that it may prevent or inhibit enzyme degradation of the molecule with subsequent loss of antibody activity in the small intestine (Tomasi & Bienenstock, 1968). Because of its high concentration in external secretions it has been postulated that IgA represents a local immunity in these secretions (Tomasi, Tan, Solomon & Prendergast, 1965; South, Warwick, Woolheim & Good, 1967). Secretory IgA is not derived from serum IgA (South, Cooper, Wollheim, Hong & Good, 1966) but is produced by plasma cells in the lamina propria of the gastro-intestinal tract and in the mucosa of the various exocrine glands (Tomasi & Bienenstock, 1968). "Secretory piece" is not manufactured by plasma cells but by certain epithelial cells (Tomasi, 1967; Heremans & Crabbé, 1968) and it is thought that the IgA, as it passes between the epithelial cells to the external secretions

acquires the secretory piece (Tomasi & Bienenstock, 1968). Since IgA has only been recently isolated in the bovine, little is known of its antibody activity. In nasal secretions, antibody against Pasteurella haemolytica has been demonstrated as being mainly of the IgA class (Duncan, Wilkie & Winter, 1971) and in Vibrio foetus infection, vaginal mucous antibodies have also been associated with IgA (Wilkie, Duncan & Winter, 1971). In the calf, nothing is yet known of its antibody activity in the small intestine, but in the neonatal pig antibody, to the O antigens of E.coli, has been found to be predominantly IgA (Porter, Noakes & Allen, 1970 a & b).

The Nature of Colostral Immunity.

Whilst Smith and his co-workers (1922 - 30) demonstrated that serum antibodies were derived from ingested colostrum, little was known of the nature of this protection other than it was immunological (Smith, 1930). Briggs (1951) in mice protection experiments, formed the opinion that the K antigens of the E.coli organism were responsible for its pathogenicity and later, when he examined samples of colostrum given to calves which had died of colibacillosis, he could not detect agglutinins to the K antigen of the E.coli strain isolated from the dead calves (Briggs, Lovell, Aschaffenburg, Bartlett, Kin, Roy, Thompson & Walker, 1951). In 1956, Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Palmer, Roy & Shillam provided supporting evidence, in that of 59 colostrum fed calves which died, 45 had received colostrum devoid of

agglutinins against the strains associated with their death. However, Smith (1962), whilst examining the aetiology of neonatal diarrhoea in calves, refuted this and did not consider that an inability to demonstrate agglutinins against either K or O antigens was a reliable guide to susceptibility to infection. Gay, McKay & Barnum (1964a) were of a similar opinion to Smith as they also were unable to detect K agglutinins. This apparent equivocation may be the result of several factors. Compared to newer techniques, direct agglutination tests are not particularly sensitive. Ingram & Malcolmson (1970 a & b) examined 38 colostrum samples using the more sensitive passive haemagglutination and antiglobulin haemagglutination tests and were able to detect antibody against the O antigen and K antigen of 4 pathogenic E.coli strains in 100% and 97% respectively of the samples. Further, it is now known that there is considerable variation in the immunoglobulin content of colostrum, not only between individuals but also between breeds (Penhale & Christie, 1969; Kruse, 1970a). It is therefore possible that the earlier workers may have really only been measuring quantitative differences in that concentration of their negative colostrum samples may have given a positive reading. Nevertheless, in spite of this controversy which is still unresolved, it was accepted that the serum immunoglobulins were responsible for the immunity of the calf to colibacillosis and several studies have been

carried out in an attempt to quantitate these immunoglobulins in relation to the disease. Fey & Margadant (1961) had observed that 97% of calves which had died of colisepticaemia were agammaglobulinaemic. In a study of market calves Gay, Anderson, Fisher & McEwan (1965) used zinc sulphate to precipitate the serum gammaglobulins which they quantitated by monitoring the resultant turbidity and found that calves which survived had high serum levels whilst those which died of septicaemia had low levels. McEwan, Fisher & Selman (1970), having demonstrated a close correlation between gammaglobulin as measured by zinc sulphate turbidity and total serum immunoglobulin (McEwan, Fisher, Selman & Penhale, 1970), observed that in a population of market calves kept under standard conditions, the type of disease seen was directly related to serum immunoglobulin levels. Calves with high levels remained healthy, those with less had diarrhoea but survived; those with still lower levels suffered from diarrhoea and died whilst calves with little or no immunoglobulin died of septicaemia. Penhale & Christie in company with McEwan, Fisher & Selman (Penhale et al, 1970) examined the individual immunoglobulin classes in these calves and found that surviving calves had mean serum levels of 7·5 mg/ml. IgG and ·8 mg/ml. IgM whilst those dying of septicaemia had levels of ·8 mg/ml. IgG and ·2 mg/ml. IgM. Calves dying with non-septicaemic diarrhoea had intermediate levels of

5·0 mg/ml. IgG and ·6 mg/ml. IgM. There was a significant difference between the level in surviving and septicaemic calves. From the evidence presented it was not known which of these immunoglobulins was responsible for the protection but from earlier observations of Penhale (1965) that the antibodies to a number of O antigens from pathogenic calf strains were predominantly of the IgM class it was considered that IgM was the essential immunoglobulin. In a number of other species, there is considerable evidence that natural antibodies to O antigens of gram -ve enteric bacteria are of the IgM class (Lo Spalluto, Miller, Dorward & Fink, 1962; Pike & Schulze, 1964; Weidanz, Jackson & Landy, 1964; Michael & Rosen, 1963; Michael, Whitby & Landy, 1962; Cohen & Norins, 1968; Porter & Hill, 1970). Furthermore, Robbins, Kenny & Suter (1965) indicated that IgM antibodies were much more efficient in complement mediated bacteriolysis and as opsonins against Salmonella typhimurium than IgG antibodies. Klaus, Bennett & Jones (1969) who also examined the immunoglobulin content of calf sera, considered IgM to be the protective antibody against colibacillosis.

It is not understood how serum immunoglobulin levels are related to local enteric disease as manifested by diarrhoea. Marsh, Mebus & Underdahl (1969) were of the opinion that, when diarrhoeic, colostrum fed calves lost serum immunoglobulin into the small intestine and so presumably high serum levels would facilitate the passage of immunoglobulin into the

intestine. On the other hand, it is known that only a proportion of the colostral immunoglobulin is absorbed from the intestine and that between individuals fed at a fixed time post-partum there is little variation (Kruse, 1970b, Selman, McEwan & Fisher, 1971). Thus by measuring serum levels, one is indirectly measuring intestinal levels in that high serum levels will be reflected by high intestinal levels though the converse is not necessarily true, as calves fed colostrum after 24 hours post-partum, could have high intestinal levels which would not be reflected in high serum levels.

In man, where colostral immunoglobulins are not absorbed by the infant, IgA is the predominant immunoglobulin and it is probable that it is responsible for the local intestinal immunity as Adinolphi & Glynn, Lindsay & Milne (1966) demonstrated that colostral IgA had marked bacteriolytic activity against E.coli in the presence of complement and lysozyme. In the pig, Porter, Noakes & Allen (1970 a & b) are of a similar opinion that secretory IgA is responsible for the local intestinal immunity in that species.

Object of Research.

As previously stated, under field conditions several forms of colibacillosis have been observed. As yet, it has not been established clearly why some calves succumb to colisepticaemia whilst others have diarrhoea. Throughout the years, evidence has been gradually accumulating that the different syndromes may be related to the immunological status of the calf. In 1921, Smith & Little observed that colostrum deprived calves very often died of septicaemia and in 1922 they examined the possibility of using pooled bovine serum as a substitute for colostrum. Small groups of calves were either given serum parenterally and/or orally and subjected to natural infection. Whilst the results were not conclusive, it did appear that the administration of serum suppressed septicaemia but had little influence on diarrhoea. Fey, Margadant, Nicolet & Hunyady (1963) injected calves intravenously and intramuscularly with 100 mls. of whey and found that of 14 calves treated 10 survived whilst 4 died with diarrhoea. None of the calves became septicaemic. Both groups of workers were using only relatively small quantities of antibody in relation to that acquired naturally by the calf and it is possible that the administration of larger quantities of whey or serum would have prevented diarrhoea. This is supported by the observations of Aschaffenburg and his co-workers (Aschaffenburg et al, 1949, 1951) that small quantities of colostral whey fed to calves prevented septicaemia, whilst

larger quantities were necessary to protect the calf against diarrhoea.

However, it is possible that both vascular and intestinal antibodies are necessary to protect the neonatal calf from colibacillosis and that these antibodies may be independent of each other in terms of the individual immunoglobulins involved. In a survey of market calves (Penhale et al 1970) there was no significant difference in the serum immunoglobulin levels of surviving calves and those which died of diarrhoea and this would support a hypothesis that vascular antibody has little influence on diarrhoea. Moreover, recent work on IgA indicating that it has a local function in the gastro-intestinal tract, would also add evidence to this hypothesis.

It was therefore decided to re-examine the nature of colostral immunity both quantitatively and qualitatively in terms of the individual immunoglobulins present in colostrum.

CHAPTER II.

As has been earlier stated in the introduction, there appears to be a direct relationship between serum antibody or immunoglobulin levels in the newborn calf and its susceptibility to colisepticaemia. Smith & Little (1922) claimed they were able to prevent natural infection by administering adult bovine serum intravenously and subcutaneously to calves in the same herd. Fey, Margadant, Nicolet & Hunyady (1963) recorded that it was possible to prevent experimental colisepticaemia in colostrum deprived calves by using colostral whey parenterally. In the light of the recent advances in our knowledge of different immunoglobulin classes and their functions, it was thought necessary to reinvestigate the protective activity of colostrum in colibacillosis more precisely, in terms of its immunoglobulin content and if possible, to establish the role of individual immunoglobulins in the immunity. Colostral whey was used in preference to adult serum because whey has very much higher levels of immunoglobulin than serum (Penhale & Christie, 1969; Klaus, Bennett & Jones, 1969). Furthermore, in colostrum, immunoglobulins are present in different proportions than in serum. Fey et al (1963) ensured that the whey in their experiment possessed specific activity against their challenge E.coli strain by vaccinating the donor cows with this serotype. In the present experiment, this was considered unnecessary as various workers have demonstrated that colostrum and serum

from normal cows contains specific antibodies to many pathogenic serotypes of E.coli (Briggs et al, 1951; Penhale, 1965; Ingram & Malcolmson, 1970 a & b), presumably because they have acquired natural immunity by contact with pathogenic E.coli present in their environment. The present study therefore describes the influence of parenterally administered whey on the development of experimental colibacillosis with the object of further elucidating the aetiology and pathogenesis of this syndrome.

MATERIALS & METHODS.

Calves.

Newborn unsuckled Ayrshire bull calves were collected as soon as possible after birth from a closed herd. They were weighed and placed in freshly disinfected pens. Prior to injection with whey, a serum sample was obtained from each calf and subsequently checked by immunoelectrophoresis and single radial immunodiffusion to ensure that it was colostrum deprived. The calves were injected with varying doses of whey intraperitoneally at the left sublumbar fossa. Two hours later, they were given a culture of a septicaemic strain of E.coli orally. Control calves only received the E.coli culture. Calves were fed by a bottle and teat, milk from cows in late lactation, a maximum of 1·5 litres per 30 kg. body weight twice daily. No attempt was made to feed calves which were disinclined to suckle. Milk was not fed until after the colostral whey and E.coli culture had been administered.

The calves were kept under observation and twice daily, pulse, rectal temperature and respiratory rates were recorded. At the same time, heparinised and clotted blood samples were taken aseptically from the jugular vein using evacuated test tubes (Vacutainer - Becton Dickinson). Changes in haematocrit values were monitored. A faeces sample was obtained from the rectum daily and dried to constant weight. A calf was considered to be diarrhoeic if the faecal dry matter was less than 10%.

Post mortem examination was carried out as soon after death as practicable; on occasions when there was any delay, the cadavers were kept in cold storage. Sterile swab samples were taken from a number of sites, including the intestine, for bacteriological examination (Appendix I).

Preparation of Colostral Whey.

Colostrum, taken at the first postpartum milking, was collected from several herds around Edinburgh and from a herd in Northern Ireland. The fat was removed by centrifugation at 820g for 1 hour, followed by filtration through a coarse nylon filter (Maxa Blow Ltd.). Casein was clotted by adding essence of rennet at the rate of 4 ml. per litre of colostrum. After clot retraction, the various whey samples were pooled, centrifuged at 35,000g for 1 hour and the supernatant dialysed against buffered physiological saline to pH 7·3. It was then filtered through cellulose membranes (Millipore Ltd.) of diminishing pore size until it was finally made bacteriologically sterile by passage through a 0·22 μ membrane. The whey was

divided into aliquots of 100 ml. and stored at -20°C. Samples were retained for serological and immunological analysis.

Initially, whey was given intravenously, but the colostrum was found to be heavily contaminated when collected from the farms and, in spite of bacteriological sterilisation, caused sudden collapse and death in two calves when given by this route. This toxicity was presumed to be due to the presence of endotoxin or other bacterial products. The intra-peritoneal route was therefore adopted as an alternative because, though calves still showed varying degrees of shock, they recovered quite quickly, usually within 2 hours, at which time they were given the E.coli culture.

Absorption of Whey from the Peritoneum.

In the early stages of the experiment, the degree of absorption of immunoglobulin from the peritoneum was measured in four randomly selected calves which had been given different doses of whey. Pre-injection and 12 hour post injection serum samples were examined for antibody activity and IgG and IgM content.

Measurement of Haematocrit Changes.

The haematocrit value of each heparinised blood sample was measured by a micro-technique (Hawksley). Sealed capillary tubes containing the samples were centrifuged at 14,000 rpm for 5 minutes. Each sample was tested in duplicate and the packed cell volume measured directly on a microhaematocrit

scale. The difference between the two samples was never more than 1% and thus a change of 2% or more between daily samples was considered to be significant. Because of considerable individual variation in the initial haematocrit values of the calves it was not possible to directly compare these values and so the percentage haematocrit change of each daily sample (Ha) compared with the first sample (Hp), taken prior to the injection of whey, was calculated as follows:-

$$\% \text{ haematocrit change} = \frac{Ha - Hp}{Hp} \times 100$$

Escherichia Coli Culture.

E.coli serotype O78K80(B) was used throughout the present experiments. As previously stated, this serotype is a septicaemic one and appears to have special pathogenicity for the calf. The strain used in these experiments had been isolated from calves experimentally infected by Penhale (1965) and stored by him. It was passaged through a control calf to ensure that there was no loss of virulence and re-isolated from the organs and peripheral blood of that calf. Stock cultures were maintained by transfer on Dorset egg slopes. Weekly subculture to MacConkey agar plates was carried out and after overnight incubation these were stored at 4°C. When a calf became available, growth from a smooth colony was subcultured to glucose broth and incubated for 6 hours. Each calf received 1 ml. per 10 kg. body weight of this broth.

The virulence of the organism was checked by periodic use of control calves during the course of the experiment.

Bacteriological Studies.

0·25 ml. of freshly obtained heparinised blood was spread directly on to the surface of MacConkey and sheep blood agar plates, which were incubated overnight. A number of colonies (approximately 20 per plate) were tested by slide agglutination with standard antisera against O78K80(B). Cultures of swabs taken from organs post mortem were similarly examined.

Preparation of Hyperimmune Serum against E.coli Serotypes O78K80(B) and O9K(A?).

Specific hyperimmune serum was prepared in rabbits by a multiple inoculation programme (Penhale 1965). A washed heat killed suspension of each serotype containing approximately 1×10^9 organisms per ml. was injected intravenously on alternate days, the dose rising from 0·1 ml. to a final volume of 1 ml. The rabbits were bled 10 days after the final injection.

Serology.

Antibody activity in the whey and serum samples was assessed by indirect haemagglutination using chicken erythrocytes sensitised with O antigens of two E.coli serotypes O78K80(B) and O9K(A?). In the titration of antibody, a 1% suspension of sensitised erythrocytes was added to serial two fold dilutions of whey and serum using microtiter equipment (Cooke Engineering Co.). The whey and serum samples were

inactivated by heating at 56°C for 60 minutes. Non-specific anti-erythrocyte activity was checked by incubating serum and whey samples with non-sensitised cells.

Preparation of O antigens of E.coli Serotypes.

Freeze dried organisms of each serotype were used to inoculate nutrient broths which were incubated at 37°C for 24 hours. The broth cultures were then transferred to Roux flasks, again incubated at 37°C for a further 24 hours after which the organisms were harvested. They were washed three times with 0·85 saline. Alkaline extracts were prepared from each serotype according to the technique of Penhale (1965).

Sensitisation of Chicken Erythrocytes.

Erythrocytes obtained from White Leghorn chickens were used in the tests. These were washed three times in normal saline. 0·1 ml. of packed cells were resuspended in 10 ml. of 0.85% saline and quantities of extract between 0·1 and 0·5 ml. depending on their effectiveness were added. The optimum quantity of extract was determined by titrating cells which had been incubated with various quantities of extract against the homologous E.coli rabbit antisera. It was found that at higher concentration the extract caused lysis of the chicken erythrocytes during sensitisation. The sensitisation was carried out according to the method of Buxton (1959). After sensitisation, the chicken erythrocytes were washed three times in 0·85% saline to remove free extract and the cells were then resuspended in 0·85% saline as a 1% solution.

Immunological Studies.

Preparation of Immunoglobulins.

Purified IgM and IgG, prepared from adult bovine serum, were used for specific antiserum production and as standards in the quantitative assay of immunoglobulins in serum and whey. IgG was prepared by fractionation of pooled adult bovine serum by ion exchange chromatography on D.E.A.E. cellulose (Whatman D.E.52) using 0·01M phosphate buffer pH 7·5 according to the technique of Penhale & Christie (1969).

IgM was prepared by first precipitating it as a euglobulin by diluting serum with distilled water (1:14). The precipitate was resuspended in a small quantity of 0·1M tris HCl buffer pH 8·0 containing 1M NaCl and fractionated by a gel filtration on a column (90 x 4 cm.) of Sephadex G200 (Pharmacia Ltd., London). The first half of the 19S peak was concentrated by dialysis against polyethylene glycol (Carbowax 6,000 Union Carbide Ltd., Southampton) and recycled on Sephadex until a single symmetrical peak was obtained. This was dialysed for 24 hours against distilled water at 4°C and finally freeze dried.

On immunoelectrophoresis, the IgG and IgM preparations were each found to give a single precipitation line typical in appearance and position to IgG and IgM respectively (Fig.3). The total protein content of the standard immunoglobulin solutions were determined by nitrogen analysis using the micro-Kjeldahl technique described by Chibnall, Rees & Williams (1943).

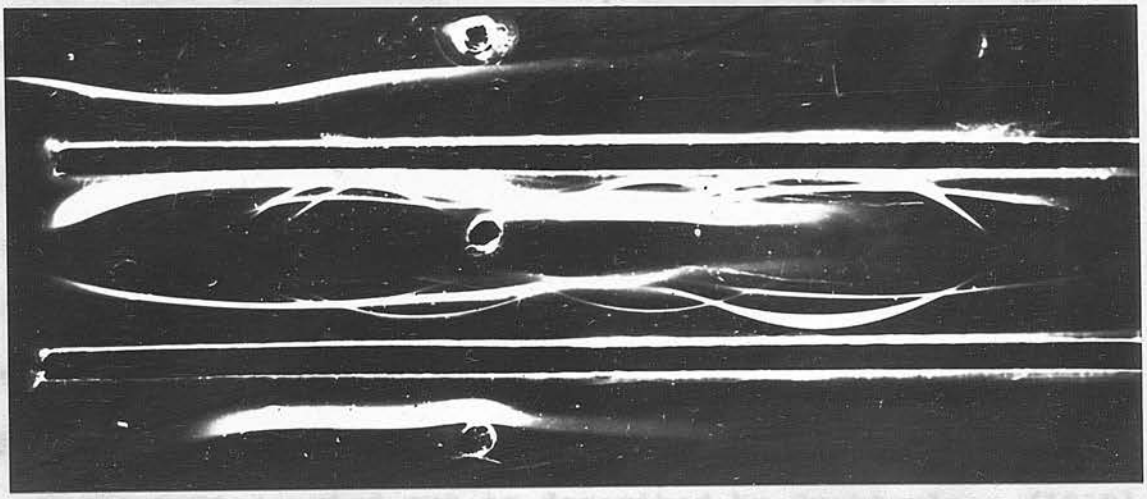

FIGURE 3.

Immunoelectrophoresis of purified IgG and IgM.

upper well IgG

centre well colostrum

lower well IgM

troughs rabbit anti-bovine colostrum serum.

Preparation of Antisera.

Antisera against bovine serum, colostrum, IgG and IgM were prepared in New Zealand white rabbits by the technique of Penhale & Christie (1969). Rabbits were given antigen emulsified in complete Freunds adjuvant. This was followed by a series of intraperitoneal and intravenous doses of antigen. The rabbits were bled ten days after the last injection.

Immunoelectrophoretic and Immunodiffusion Analyses.

These were carried out as described by Ouchterlony (1953). Immunodiffusion was performed on microscope slides using 1% agar (Difco Noble) in normal saline.

Immunoelectrophoresis was carried out on a 8 x 8 cm. glass plate using 1% agar (Difco Noble) with barbitone acetate buffer (Oxoid) at 0·05 ionic strength and pH 8·6.

Quantitative Immunoglobulin Determination.

The single radial immunodiffusion technique was used to quantitate the immunoglobulin levels. The procedure employed was similar to that of Fahey & McKelvey (1965) who had adapted the original method of Mancini, Carbonara & Heremans (1965). Prior to use, the specificity of the IgG and IgM antisera was checked by immunoelectrophoresis and immunodiffusion. On immunodiffusion, the IgG antisera reacted with only one component in bovine serum (Fig.4), and produced a typical IgG precipitin arc against pooled bovine serum on immuno-electrophoresis (Fig.5). The anti-IgM serum was not so

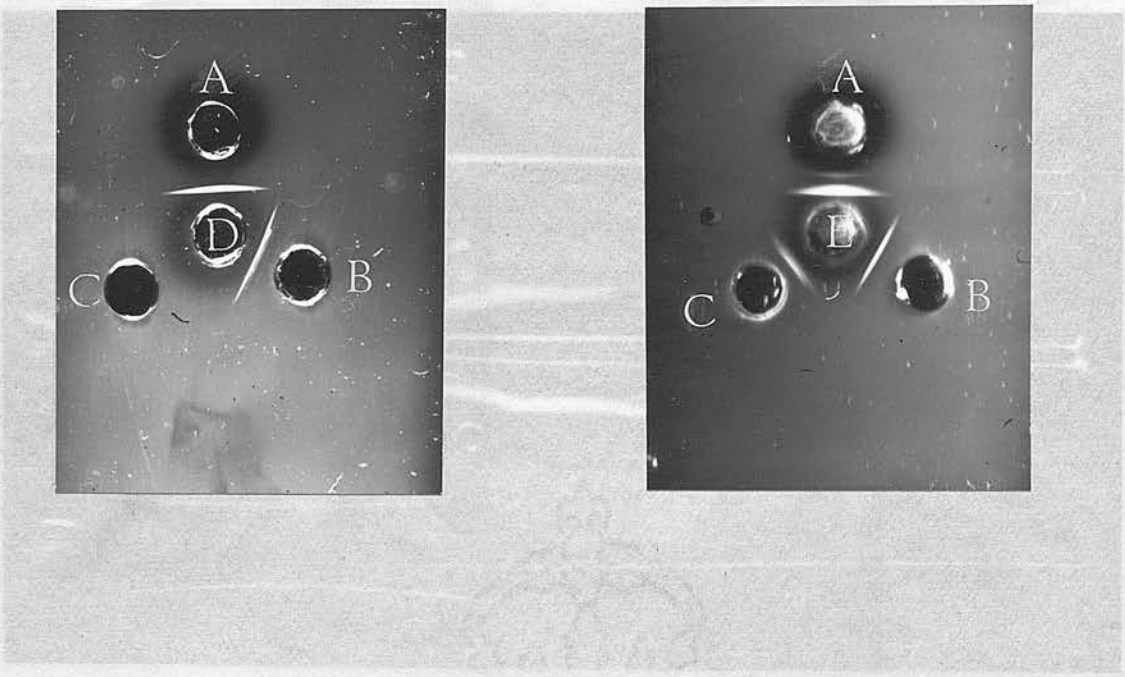

FIGURE 4

Immunodiffusion of bovine serum purified IgG and IgM against rabbit anti-IgG and anti-IgM sera.

A adult bovine serum.

B IgG

C IgM

D rabbit anti-IgG serum.

E rabbit anti-IgM serum.

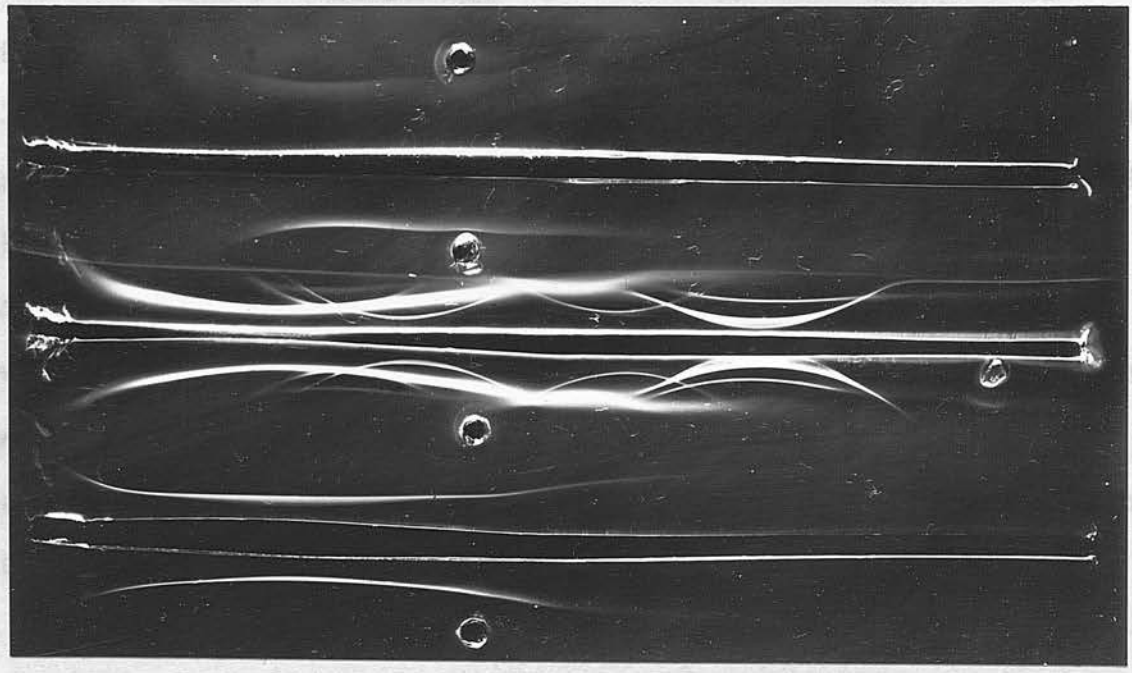

FIGURE 5

Immunoelectrophoresis of bovine serum against rabbit anti-IgG serum and absorbed anti-IgM serum.

Upper trough rabbit anti-IgM serum absorbed with IgG.

Centre trough rabbit anti-whole bovine serum.

Lower trough rabbit anti-IgG serum.

Wells contain adult bovine serum.

specific and reacted with two components in serum giving two precipitin lines, one against IgM and the other against IgG (Fig.4). The antiserum was absorbed with varying amounts of IgG preparation until a single precipitin line was obtained (Fig.5). As an extra precaution, both antisera were absorbed with precolostral or foetal calf sera. 10 mls. of 2% agar (Difco Noble) and an equal volume of diluted specific antiserum were mixed and poured on to a clean level 10 cm. x 10 cm. glass plate. Using a plastic template (Fig.6) 36 wells of equal size were cut in the hardened agar. It was thus possible to have 30 test samples, together with 6 serial dilutions of the purified immunoglobulin standard on each plate (Fig.7). Later, serum of known immunoglobulin content was used as a standard for IgG estimations whilst whey was similarly used for IgM estimations. The plates, after incubation in a humidity chamber at $4^{\circ}C$ for 24 hours in the case of IgG and 48 hours for IgM, were washed, dried and stained with a 0·5% amido Schwartz 10B in methanol glacial acetic acid 9:1. The precipitin ring diameters were measured using a magnified scale (Matchless Machines Ltd., Surrey).

Total Protein Estimation of Whey.

This was done by Biuret analysis.

Statistical Analysis.

The results were examined statistically by Probit analysis and by the method of Dixon & Wood (1948).

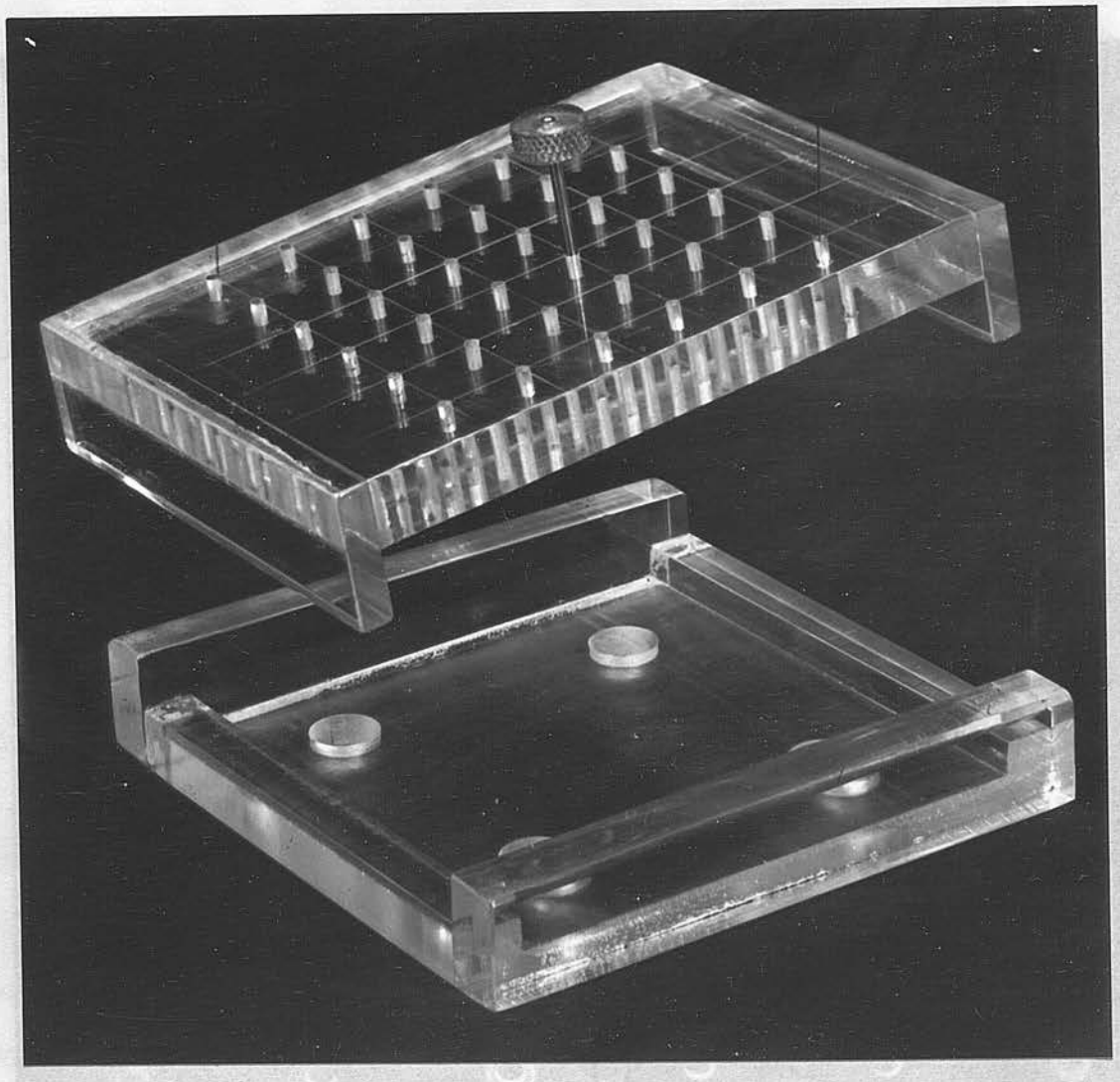

FIGURE 6

Template for single radial immunodiffusion.

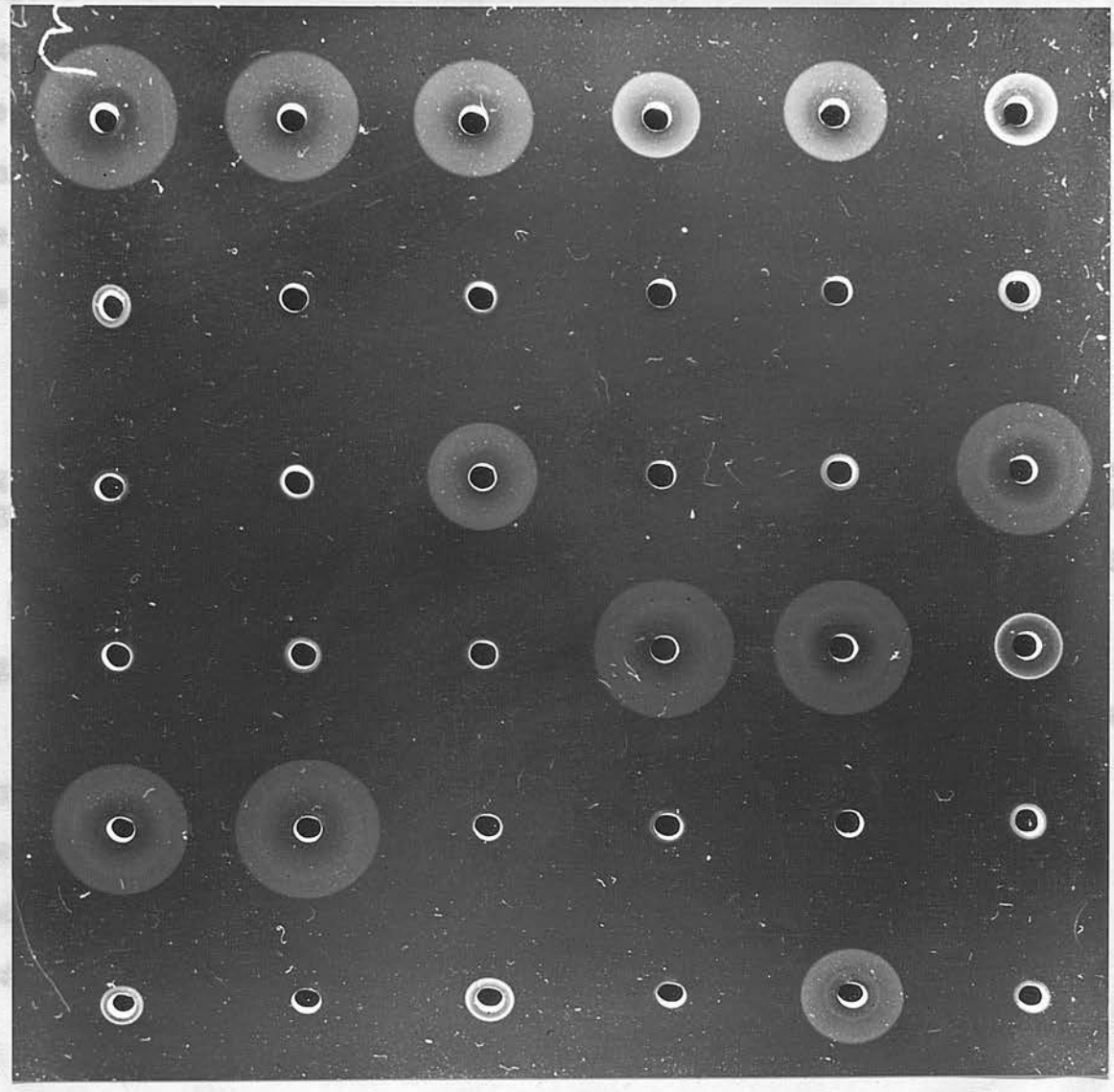

FIGURE 7

Single radial immunodiffusion plate (IgG).

Standard dilutions of IgG on top row;
dilutions 1/16 & 1/8 are reversed.

RESULTS

The immunochemical and serological analyses of the colostral whey.

Examination of the immunoelectrophoretic pattern of the whey (Fig.[8]) showed that in the gamma region, the predominant component was IgG1. In addition, IgM and IgA were also present. The quantitative results are summarised in Table I where it also can be seen that, as adjudged by indirect haemagglutination, the whey had retained antibody activity to the O antigens of the two serotypes of *E.coli* against which it was tested.

Table I.

Analysis of Colostral Whey

Total protein mg. per ml.	IgG mg. per ml.	IgM mg. per ml.	Haemagglutination Titre O9	O78
71	24	4	128	64

Absorption of whey from the peritoneum.

From Table II it can be seen that, in each case, there was a post-injection increase of serum immunoglobulin levels accompanied by a significant rise in haemagglutination titre indicating that effective absorption of both immunoglobulins had occurred from the peritoneum.

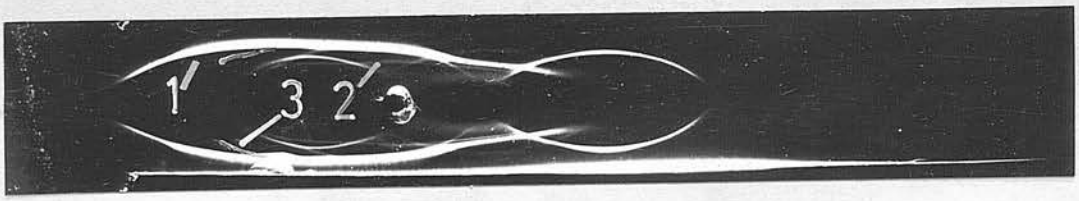

FIGURE 8

Immunoelectrophoresis of the colostral whey pool.

1 = IgG

2 = IgM

3 = IgA

Trough - rabbit anti bovine serum.

Table II.

Absorption of Whey from the Peritoneum

Calf No.	Dose g. per 30 kg. bodyweight		Pre-injection serum levels mg. per ml.		HA titre O78	12 hr. post injection serum levels mg. per ml.		HA titre O78
	IgG	IgM	IgG	IgM		IgG	IgM	
8	3·0	0·5	0	0	0	3·1	0·43	16
9	1·5	0·25	0	0	0	1·6	0	8
10	0·95	0·125	0	0	0	0·35	0	8
12	2·7	0·45	0	0	0	0·24	0	4

HA = Haemagglutination.

The Effect of Colostral Whey administration.

Only calves which were found to have no immunoglobulin in their initial pre-injection serum were used in the experiment. In Fig.9 the typical immunoelectrophoretic pattern of a colostrum fed calf is compared to that of a deprived calf. Colostrum-deprived calf serum is usually agammaglobulinaemic but occasionally may contain immunoglobulin which has been produced in utero (Klaus, Bennett & Jones, 1969).

The results of the administration intraperitoneally of whey in various quantities are summarised in Fig.10 in which clinical findings are related to the IgM and IgG content of the dose of whey injected. After the first few calves had been treated it became apparent that although the parenteral administration of whey above a certain level effectively excluded septicaemia it had little influence on the development

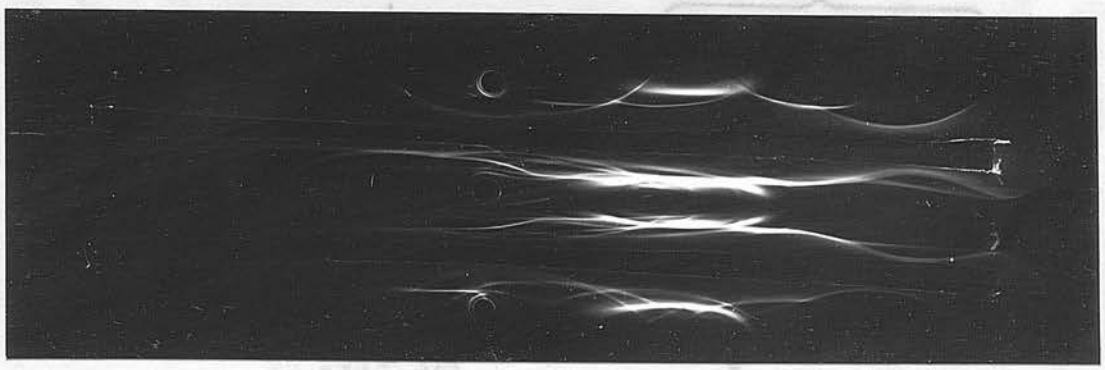

FIGURE 9

Immunoelectrophoresis of calf sera.

Upper and lower wells colostrum deprived calf.
Middle well colostrum fed calf.
Troughs rabbit anti-whole bovine serum.

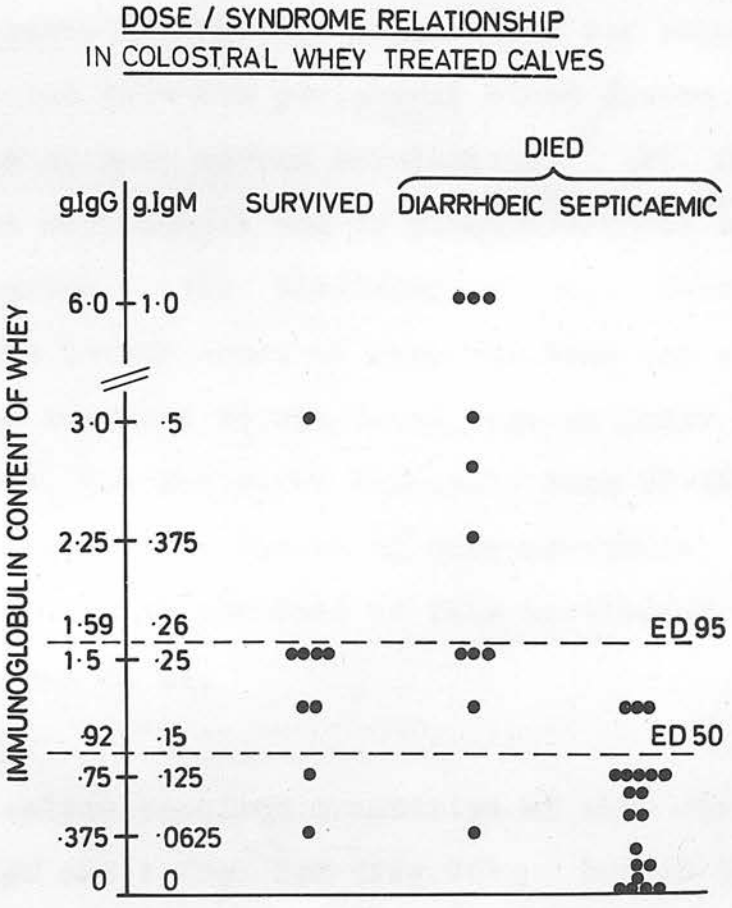

FIGURE 10

Dose - syndrome relationship in colostral whey - treated calves.
Each point represents an individual calf.

of diarrhoea and as a consequence quite a number of calves died. Clinically, calves could be divided into three groups (1) Septicaemic calves which invariably died. A calf was considered septicaemic if the test organism or any other organism was isolated from the peripheral blood during life or from the organs at post mortem examination. (2) Calves which died without septicaemia and in which diarrhoea was the most prominent feature. (3) Surviving calves. Septicaemic calves received the lowest doses of whey and when the results were statistically analysed it was found that in order to exclude septicaemia, the Estimated Effective Dose 95 (ED95) per 30 kg. body weight was a volume of whey containing 0·26g. IgM and 1·6 gms. IgG. In the case of this particular whey pool, this volume was 65 ml.

Septicaemic Calves.

Septicaemic calves received quantities of whey containing less than 0·2g. IgM and 1·25g. IgG (Fig.10). Within the group no relationship was found between the survival time and the dose of whey, e.g. one calf given only 0·04g. IgM, 0·25g. IgG (10 ml.) survived 10 days whilst one given 0·2g. IgM and 0·9g. IgG (40 ml.) lived only three days (Fig.11).

Clinically, 12 to 36 hours after challenge, there was fever, dullness and inappetence which lasted approximately 12 hours. This was followed by a variable period of improvement before the onset of septicaemia when the calves again became febrile, increasingly weak, disinclined to feed,

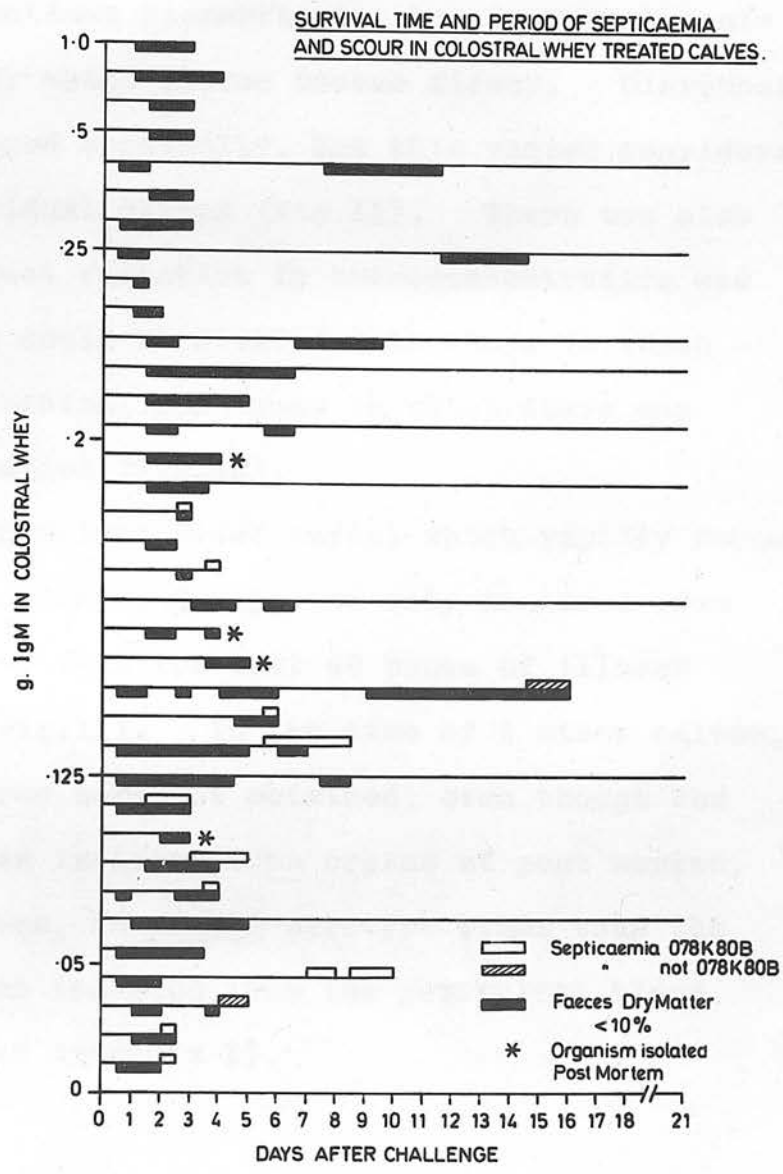

FIGURE 11

The relationship between survival time and period of septicaemia and scour in colostral whey treated calves. The solid line indicates survival time.

and eventually recumbent, after which death rapidly followed. During the initial period of depression there was invariably a severe watery, sometimes haemorrhagic, but transient, post-meconial scour, after which faeces became firmer. Diarrhoea again usually developed terminally, but this varied considerably in severity in individual calves (Fig.11). There was also considerable individual variation in haemoconcentration and basically the calves could be divided into those in which P.C.V. changes were minimal and those in which there was marked haemoconcentration (Fig.12).

Unlike untreated calves (vide infra) which rapidly became septicaemic after challenge, E.coli was only isolated from the peripheral blood within the last 48 hours of illness except in 2 calves (Fig.11). In the case of 4 other calves, positive blood cultures were not obtained, even though the challenge organism was isolated from organs at post mortem, and in 2 further calves, an E.coli serotype other than the challenge organism was isolated from the peripheral blood and organs (Fig.11 and Appendix I).

Diarrhoeic Calves.

Calves which died without becoming septicaemic, received doses of whey comparable to those given to calves which survived. (Fig.10). Clinically, the outstanding feature of these calves was the occurrence of a profuse, watery diarrhoea which commenced immediately after passage of the meconium and continued without remission until death (Fig.11). Survival

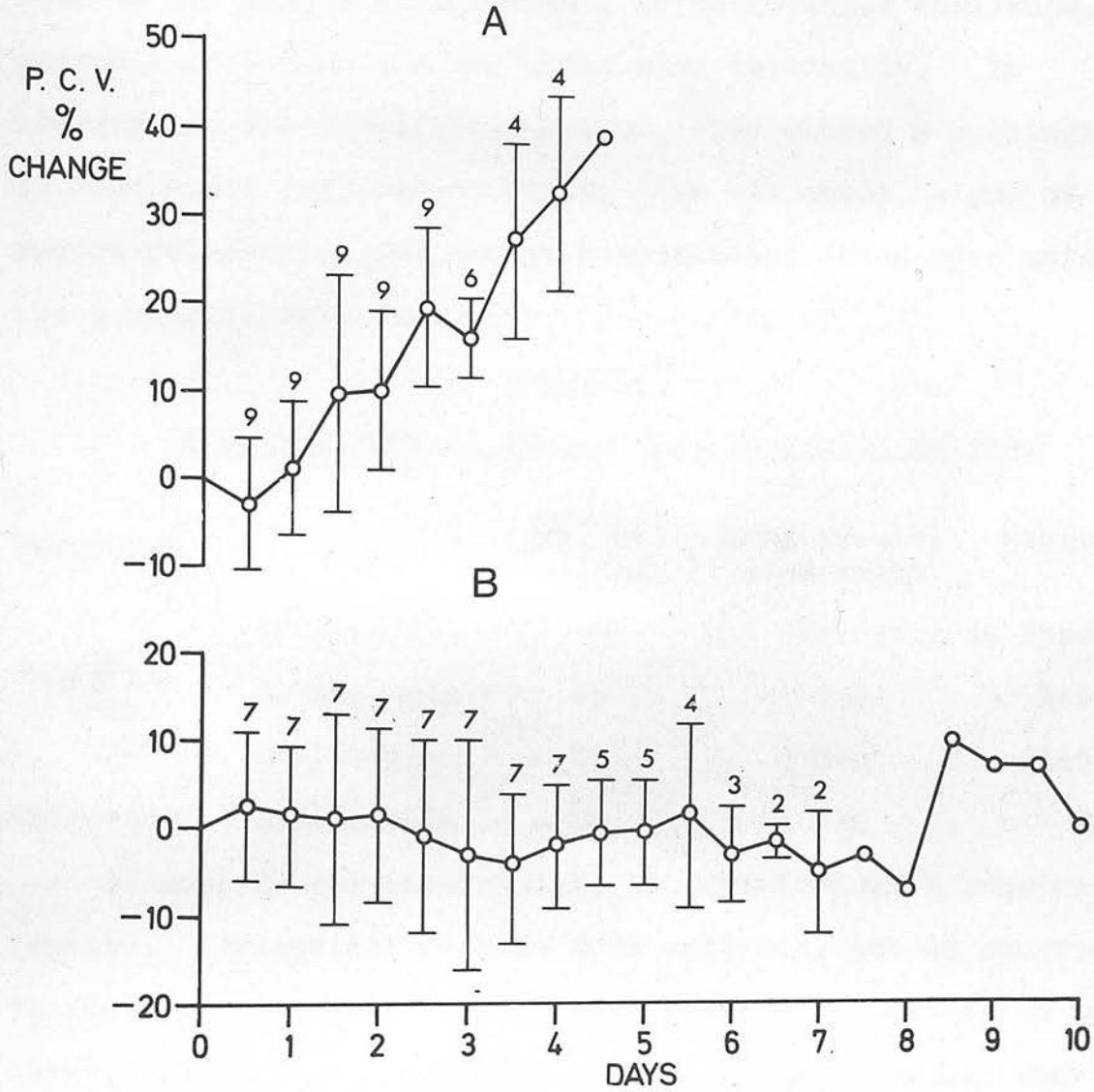

FIGURE 12

Daily changes with standard deviation (S.D.) in packed cell volume of septicaemic calves. A marked haemoconcentration. B minimal haemoconcentration. Figures above S.D. are numbers sampled.

time was extremely short, calves with one exception dying within 3 - 5 days which was less than the mean survival time of septicaemic calves (Table III). After an initial pyrexia, seen in all calves approximately 24 hours after challenge, a raised temperature was not noted even terminally. In contrast to the septicaemic calves, they showed a willingness to feed until just before death. In all cases, signs of severe dehydration and marked haemoconcentration were evident for a considerable time before death (Fig.13).

TABLE III

Survival Time of Treated and Untreated Calves.

Treatment	Group	No. of calves	Mean survival time (days)	Range (days)
	Survivors	9	All surviving at three weeks	
Colostral whey	Septicaemic	16	5·4	2·5 - 16
	Diarrhoeic	11	3·0	1·5 - 5
Untreated	Septicaemic	4	2·1	1 - 3

At post mortem examination, dehydration was a constant feature. Intestinal changes were variable, but in contrast to septicaemic calves there was an absence of congestion in the liver, lungs, kidney and spleen. On bacteriological examination no organisms were isolated from any tissue except the mesenteric lymph nodes where variable numbers of mucoid types of E.coli were found. Throughout the proximal small intestine of these calves, E.coli generally of the mucoid type, were isolated in

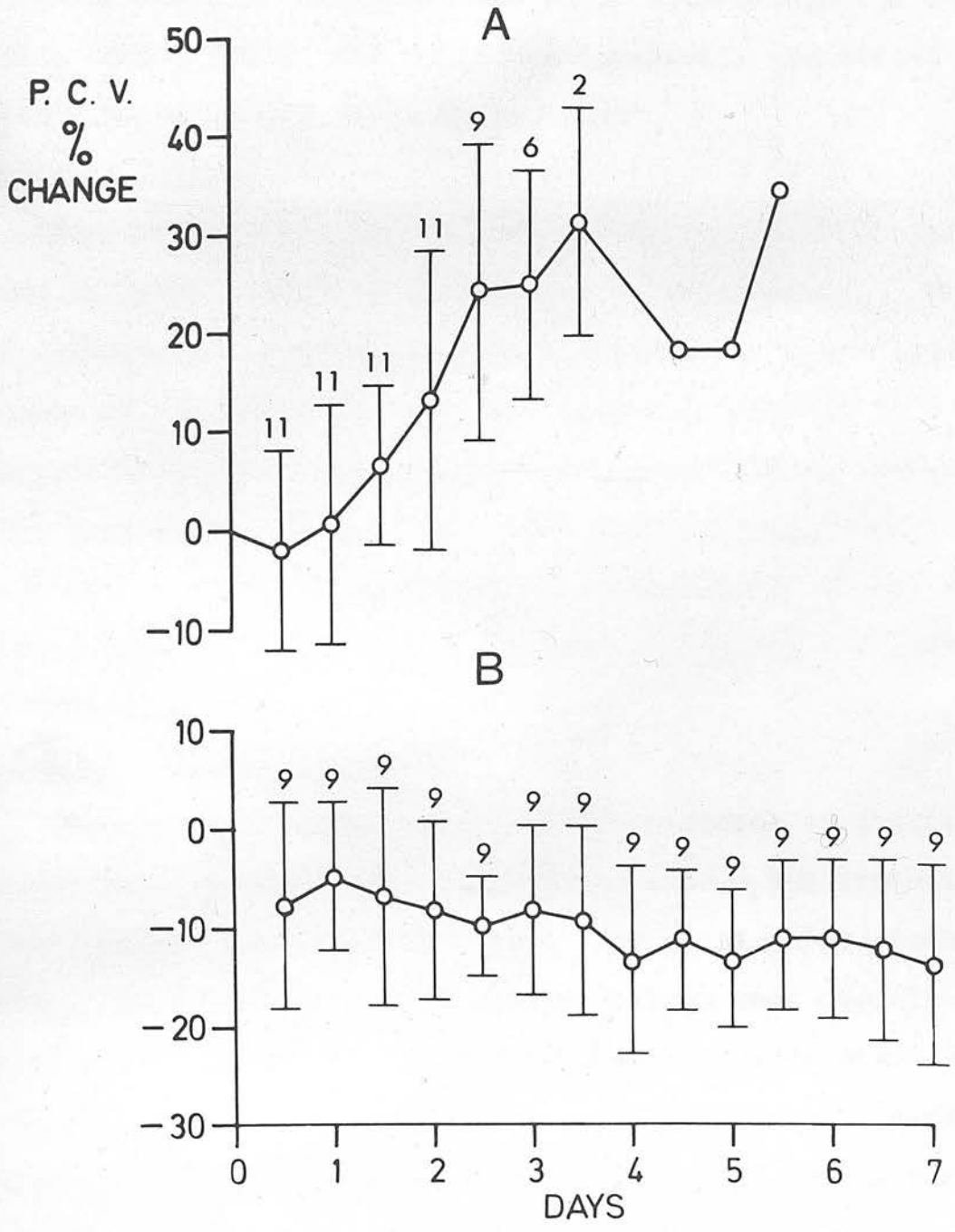

FIGURE 13

Daily changes with standard deviation (S.D.) in packed cell volume of A diarrhoeic calves. B surviving calves. Above S.D. are numbers sampled.

large numbers (Appendix I). Several of these strains of E.coli have been shown to belong to the known enterotoxigenic strains "B42", "B85", "B117" and "B111" as originally identified by Smith & Halls, 1967a (W.J. Sojka, 1970).

Surviving Calves.

Surviving calves showed some similar clinical features to those in Group I despite the absence of septicaemia. There was initial dullness and diarrhoea followed by rapid recovery. In some calves there was a second period of pyrexia at 5 to 8 days together with diarrhoea (Fig.11) from which calves recovered in a few days and at 21 days were thriving (Fig.14). At 1 month, these calves were slaughtered and no abnormalities were observed at post mortem examination. On bacteriological examination all tissues were sterile.

Untreated Control Calves.

The experimental serotype was administered to 4 calves which did not receive whey (Fig.15). These showed the typical picture of the disease described by others (Fey et al, 1963; Penhale, 1965). In contrast to whey treated calves they rapidly became septicaemic. This was rapidly followed by prostration and death occurred in all cases within 72 hours (Table III). Haemo-concentration was variable (Fig.16). At post mortem examination, ma croscopic changes typical of septicaemia were observed. There were haemorrhages in the spleen, kidneys and cardiac musculature, particularly around coronary vessels and valves (Fig.17) and the lungs were congested. The challenge organism was isolated from all

FIGURE 14

Surviving calves at 3 weeks old.

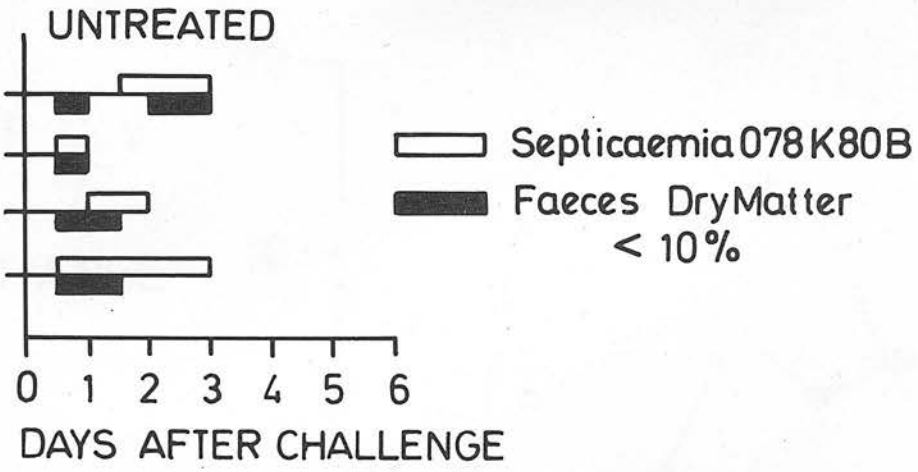

FIGURE 15

The relationship between survival time and periods of septicaemia and scour in untreated control calves.
The solid line indicates survival time.

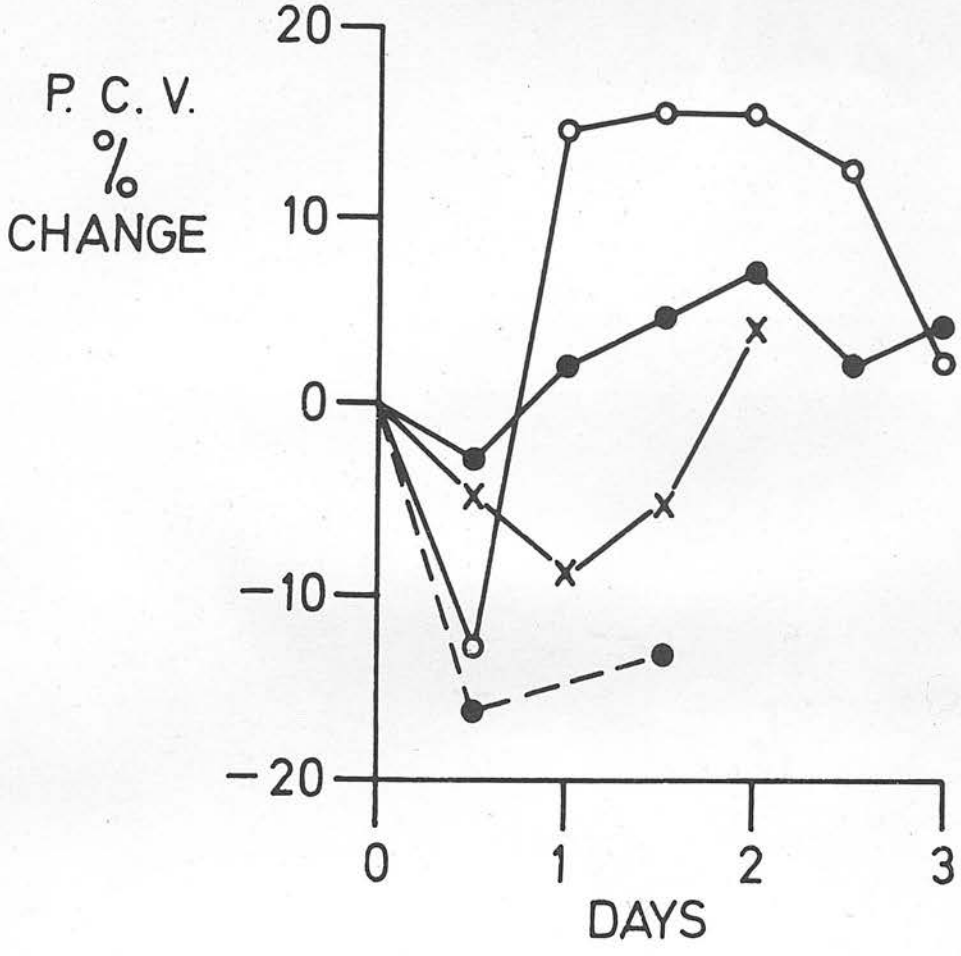

FIGURE 16

Daily changes in packed cell volume of individual untreated control calves.

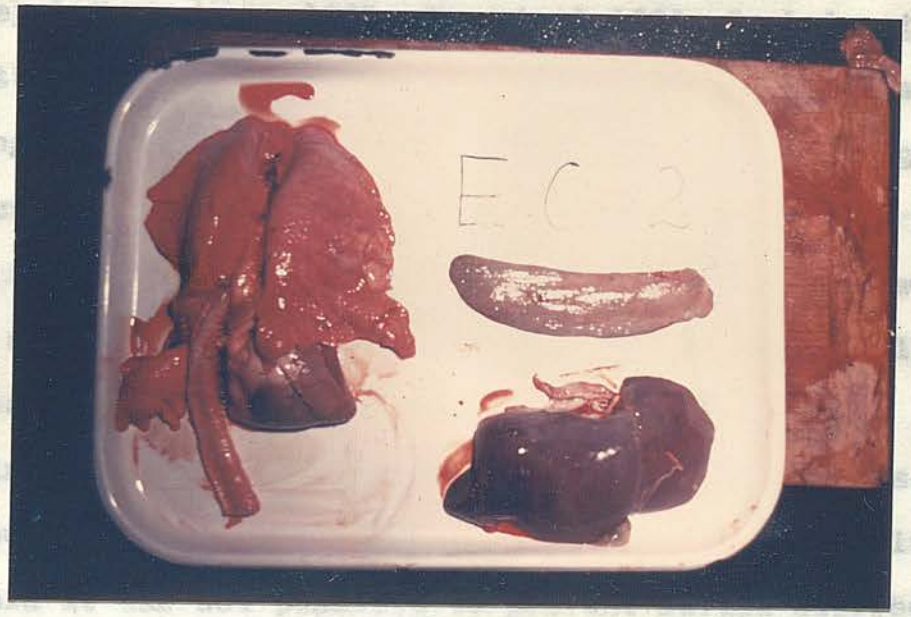

FIGURE 17

Organs from septicaemic calves.

sites in pure culture (Appendix I).

DISCUSSION.

The administration of whey parenterally, at a suitable level, clearly divided the neonatal scour complex into two distinct syndromes. The results are in agreement with those of Fey et al (1963) who, using whey intravenously and intramuscularly in doses of 50 - 100 ml. also found it possible to protect calves against septicaemia but not against diarrhoea. This finding confirms the observations of a number of workers that under natural conditions, the character of the spontaneous disease occurring in a particular calf depends on its immune state (Fey & Margadant, 1961; Gay et al 1965; Penhale et al 1970). These authors showed that calves which developed septicaemia were hypogammaglobulinaemic, whereas those which had higher serum immunoglobulin levels did not develop septicaemia but suffered from severe diarrhoea. In the field, these two distinct syndromes can occur either separately or more likely, combined as a septicaemic - enteric complex.

During the present experiment, in spite of giving some calves four times the quantity of whey necessary to exclude septicaemia it was not possible to prevent severe diarrhoea. An explanation for this failure may have been the inability of the whey to reach the lumen of the gastro-intestinal tract in effective quantities. This would suggest that under natural circumstances, when a calf acquires colostrum orally, the colostrum may have a dual protective function; locally within

the gastro-intestinal tract and systemically following absorption. In this connection a passive local protective effect has been indicated in transmissible gastro-enteritis in piglets. Hooper & Haelterman (1966) demonstrated that it was necessary that the antibody supplied via the colostrum and milk should be present in the gut, and they referred to this as "lactogenic immunity". Because of this requirement, parenteral administration of antiserum was ineffective in controlling the disease. Noble (1964) reinforced this hypothesis by successfully protecting piglets by feeding them serum taken from recovered animals.

In the light of recent work on the biological function and structures of immunoglobulins, it is likely that these two immune systems may be mediated by different classes of immunoglobulin. Adinolfi, Glynn, Lindsay & Milne (1966) demonstrated that in the human, antibody to E.coli in serum was of the IgM class, whereas antibody in colostrum was of the IgA class and Porter, Noakes & Allen (1970a) reported a similar finding in the pig.

It is possible that the occurrence of diarrhoea had an important bearing on the survival time of septicaemic calves and the considerable variation in severity as reflected by differences in haemoconcentration may have accounted for the inability to establish a dose/survival time relationship. Marsh, Mebus & Underdahl (1969) are of the opinion that there is a loss of plasma immunoglobulins into the intestine during scouring. Depending upon the severity, there is therefore likely to be a

variable loss of circulating immunoglobulins which would reduce the survival period accordingly. Studies on circulating IgG in the calf (MacDougall & Mulligan, 1969) showing that during scouring the half life is significantly reduced, add further evidence to this possibility, and although IgM catabolism was not investigated, it is possible that it is similarly affected.

In view of the acuteness of the condition of the diarrhoeic non-septicaemic calves and the fact that dehydration alone did not seem entirely to account for the marked deterioration observed, this syndrome bears some similarities to that described as enterotoxaemia by Gay, McKay & Barnum (1964b). In many ways, clinically, these calves resembled the untreated septicaemic calves, although organisms could not be isolated from the blood or tissues post mortem. The marked haemo-concentration, a feature of these calves, also suggests that they might be influenced by endotoxin derived from the gastro-intestinal tract. It would appear that this syndrome occurred spontaneously and was not associated with the administered serotype O78K80(B), since this strain was never isolated in these cases from any tissues and was rarely found in the gastro-intestinal tract at post mortem examination. It is more likely to be associated with the presence of large numbers of E.coli of the mucoid type which were found in the small intestine and which have been shown to belong to entero-toxigenic serotypes, as adjudged by their ability to cause

dilation of ligated loops.

This study clearly emphasises the comprehensive nature of the passive immunity provided by colostrum and suggests that it may embrace at least two systems, each adapted to confer immunological protection on a particular body compartment.

CHAPTER III.

In the previous chapter it was demonstrated that 65 ml. of a colostral whey pool which contained ·26g. IgM and 1·5g. IgG could prevent septicaemia when given intraperitoneally.

Throughout the literature there is considerable evidence that IgM appears to be the antibody most active against E.coli. Penhale (1965) reported that IgM was the predominant antibody against O antigens of E.coli in bovine serum. Similarly, Adinolfi, Glynn, Lindsay & Milne (1966) and Porter & Hill (1970) confirmed that in human and pig sera respectively, antibacterial antibodies against E.coli were of the IgM class. In a study of the plasma immunoglobulin levels of market calves (Penhale et al 1970), it was suggested that IgM was the immune component responsible for natural prophylaxis to colisepticaemia as calves in which IgM was not detected invariably died even though in some cases IgG was present in the sera. These findings are further supported by the fact that in other species, the bulk of the natural serum antibody to gram negative enterobacterial antigens is IgM (Michael, Whitby & Landy, 1962; Michael & Rosen, 1963; Cohen & Norins, 1968).

There is little positive evidence that IgG contains antibody to E.coli. Under field conditions, Lotan, Berman, Tadmor & Perk (1964) reported that a serum IgG fraction prepared by alcohol precipitation improved the percentage survival in neonatal calves in the first year of use but in the second year, had no effect. Watt (1967) stated that a serum fraction, obtained after ammonium sulphate precipitation

protected calves against colibacillosis and he attributed the prophylactic effect to the presence of IgG in the fraction.

Since IgM and IgG are the principal immunoglobulins of bovine serum (Penhale & Christie, 1969; Klaus, Bennett & Jones, 1969; Mach & Pahud, 1971) it was decided to administer these immunoglobulins separately and in excess of that found in the ED95 dose of whey and establish, if possible, which was the effective antibody class.

MATERIALS & METHODS.

The experimental protocol was similar to that described in Chapter II excepting that the calves were injected intraperitoneally with quantities of the individual immunoglobulin fractions of whey.

Preparation of Immunoglobulin Fractions.

The immunoglobulin fractions were prepared from the same pool of colostral whey as had been used previously in order to avoid any possible difference in antibody specificity which might occur with different samples.

Preparation of Immunoglobulin M.

Colostral IgM was prepared in quantities suitable for injection by diluting filtered whey with 14 volumes of distilled water. After 24 hours the precipitate was recovered using a continuous flow rotor (M.S.E. Ltd., London) and dissolved in a suitable quantity of 0·1M tris HCl buffer (pH 7·9) containing 1M NaCl. Approximately 20 volumes of buffer were used for each litre of original whey. Insoluble

material was removed by high speed centrifugation and the supernatant was fractionated on a 90 cm. x 64 sq.cm. column of Sephadex G 200 (Pharmacia Ltd.) using the same buffer as above and with a pump speed of 80 ml./hour. To reduce contamination with other whey components only the first half of the exclusion peak was harvested (Fig.18). This was then dialysed against distilled water (50 volumes) for 24 hours to remove the buffer, concentrated by ultrafiltration and freeze dried. It was then redissolved in sterile phosphate buffered saline pH 7·5 prior to injection.

Preparation of Immunoglobulin G.

Colostral IgG was prepared by a batch DEAE cellulose technique (James, 1969). The immunoglobulins were first precipitated from the whey using 28% (w/v) sodium sulphate. After separation, the precipitate was redissolved in 0·15M NaCl to a final volume one quarter of that of the starting whey and dialysed overnight against a large volume (50 volumes) of phosphate buffer (pH 7·5 0·1M). DEAE cellulose (D.E.52 Whatman Ltd.) was similarly equilibrated with this phosphate buffer. Because whey contains principally IgG1, as opposed to serum which contains IgG2, it was necessary to use a buffer with a higher molarity than that used in the recovery of IgG2 from serum (Butler, 1970). The cellulose was used at approximately 1,000g. wet weight to treat the globulin obtained from 1 litre of whey. The crude globulin was added to the exchanger and stirred for approximately

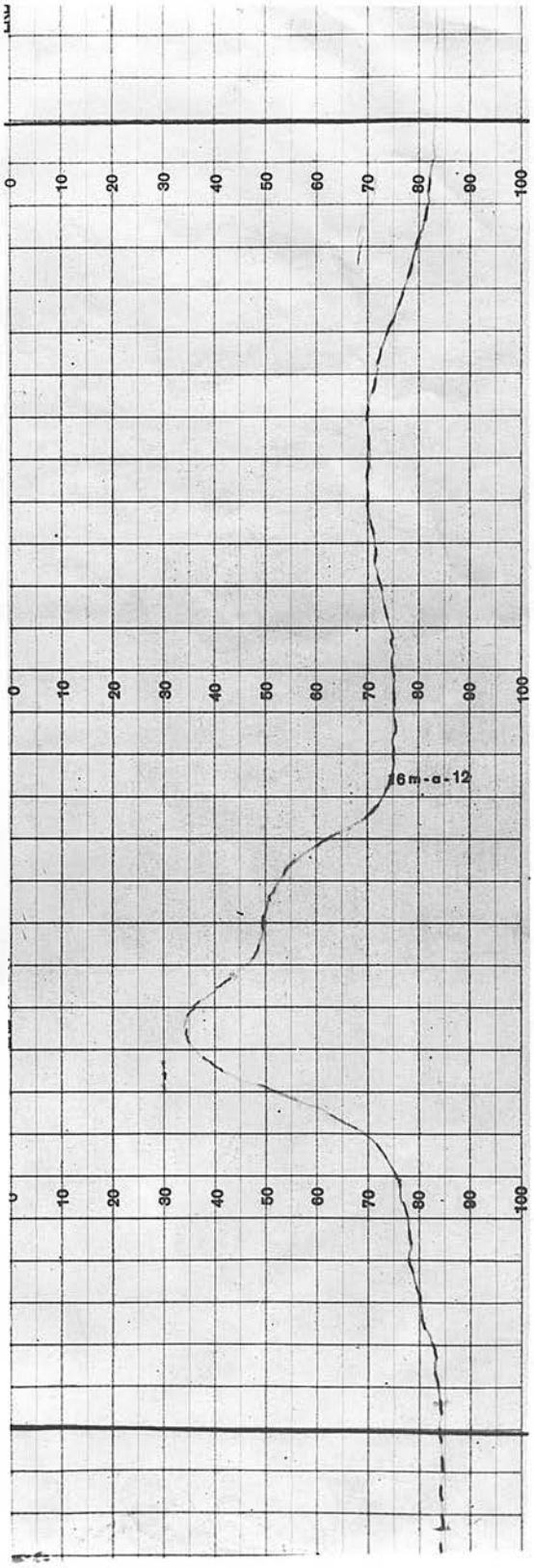

FIGURE 18

Chromatogram of colostral whey euglobulin.

10 minutes at room temperature. Phosphate buffer was then added to the thick suspension - 1 litre of buffer for 100g. exchanger - and the mixture was stirred for a further 15 minutes. The resultant slurry was filtered on a Buchner funnel. The filtrate was concentrated by ultrafiltration, dialysed against distilled water (50 volumes) for 24 hours and finally lyophilized.

RESULTS.

Analysis of the Immunoglobulin Fractions.

The purity of immunoglobulin preparations was examined by immunoelectrophoresis and the single radial diffusion test. On immunoelectrophoresis (Fig.19) the IgG was found to contain a trace of IgA and the IgM preparation a small trace of α 2 globulin, whilst the single radial diffusion test (Table IV) revealed that the IgM preparation also contained some IgG as well as other colostral proteins.

Table IV.

Analysis of Immunoglobulin Fractions Compared with Parent Whey Pool.

	Total protein mg. per ml.	IgG mg. per ml.	IgM mg. per ml.	Haemagglutination Titre. 09	078
Colostral whey	71	24	4	128	64
IgG	10	10	-	4	4
IgM	30	2	21	2,048	1,024

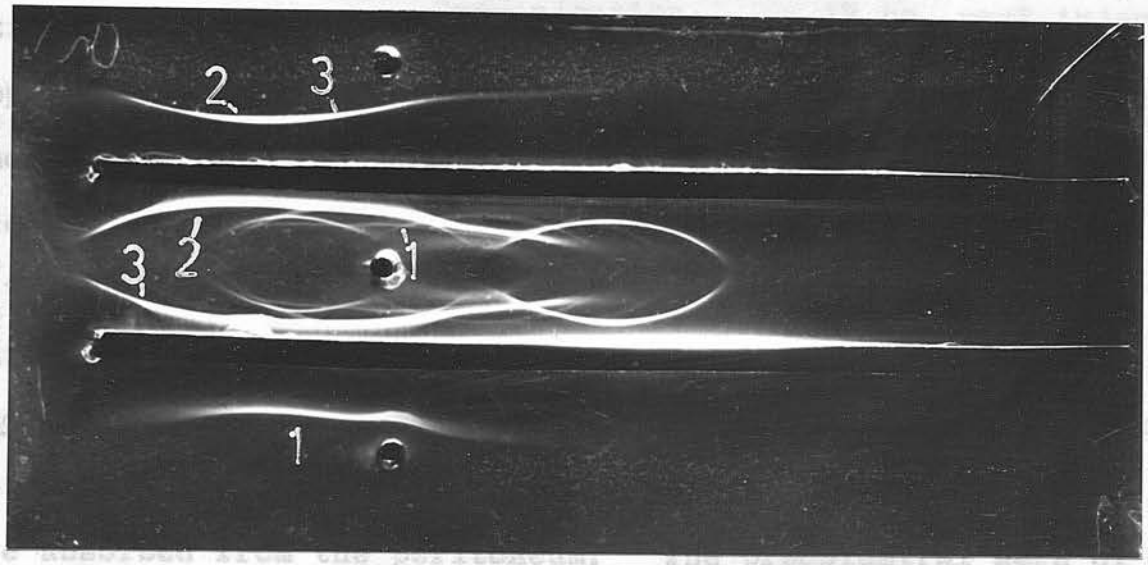

FIGURE 19

Immunoelectrophoresis of immunoglobulin fractions.

Upper well IgG

Centre well Colostrum

Lower well IgM

troughs rabbit anti-bovine serum.

1) IgM arcs. 2) IgA arcs. 3) IgG arcs.

Indirect Haemagglutination.

As can be seen in Table IV the IgG preparation contained little antibody to E.coli O antigens as judged by haemagglutination whereas antibody activity was concentrated in the IgM preparation in approximately the same proportion as the concentration of IgM. The IgM and IgG preparations were injected intraperitoneally in doses in excess of that found in the E.D.95 of whey and their effect statistically analysed against the 4 control calves in Chapter II.

Absorption of Immunoglobulin from the Peritoneum.

From Table V it can be seen that both immunoglobulins were absorbed from the peritoneum. The precolostral sera of 2 calves contained traces of immunoglobulin when measured by single radial immunodiffusion, but in each case this was considered to be of foetal origin and contained no detectable antibody activity to E.coli.

Table V.

Absorption of Immunoglobulin Fractions from the Peritoneum.

Calf No.	Dose g. per 30 kg. bodyweight		Pre-injection serum levels			12 hr. post injection serum levels		
			mg. per ml.		*HA titre O78	mg. per ml.		*HA titre O78
	IgG	IgM	IgG	IgM		IgG	IgM	
69	0	0·52	0	0·17	4	0	0·49	16
70	0	0·52	0	0	4	0	0·44	16
75	0	1·0	0	0	0	0	0·42	8
82	0	0·7	0	0	0	0	0·59	32
63	1·8	0	0	0	0	0·4	0	0
64	3·6	0	0	0	0	1·45	0	0
65	3·6	0	0	0	0	1·1	0	0
93	2·7	0	0·12	0	0	1·0	0	0
84	4·8	0	0	0	0	2·0	0	0

*HA = Haemagglutination test.

The Effect of IgM Administration.

4 calves were given IgM before challenge in quantities in excess of that found in the E.D.95 of whey (Fig.20). Although these calves died of septicaemia, the administration of IgM produced an obvious modification in the septicaemic pattern observed in untreated calves. The IgM significantly prolonged survival time ($p < 0.016$) and also delayed the onset of septicaemia ($p < 0.014$) compared with untreated calves and similarly with calves given IgG ($p < 0.014$ and $p < 0.016$ respectively). In these calves bacteraemia was only seen for a short period immediately before death (12 hours) and appeared

SURVIVAL TIME AND PERIOD OF SEPTICAEMIA AND SCOUR IN IMMUNOGLOBULIN TREATED CALVES.

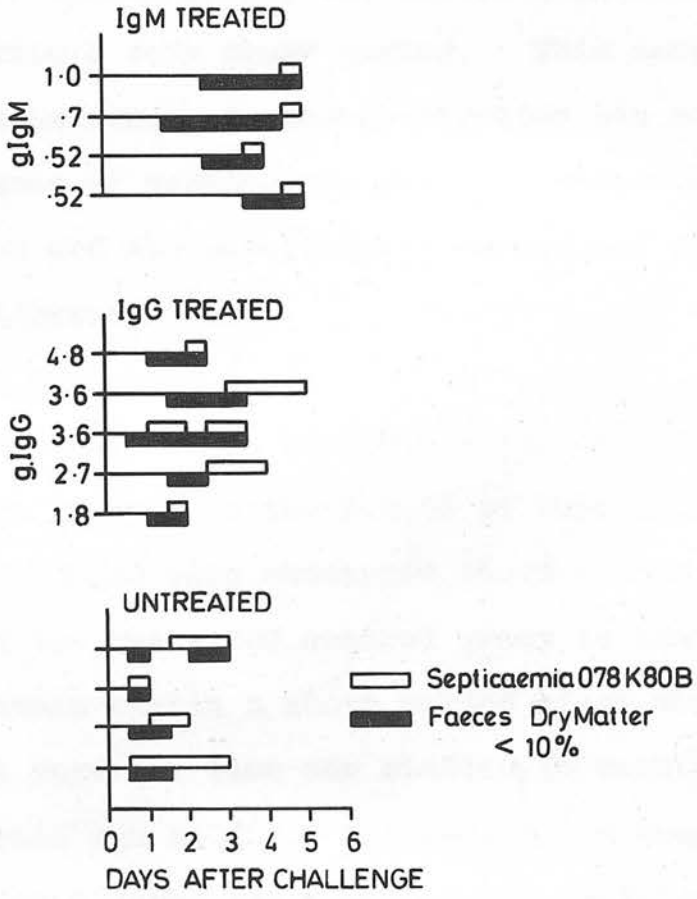

FIGURE 20

The relationship between survival time and periods of septicaemia and scour in calves receiving immunoglobulin fractions and untreated calves. The solid line indicates survival time.

to be a different character from that of the untreated calves, there being the sudden influx of large numbers of organisms (approximately 10,000 per ml.), as opposed to the more gradual increase in the former group (Appendix II). This bacteraemia was accompanied by severe shock, the calves' condition rapidly deteriorating within a very short period. This syndrome was seen in all these calves. Haemoconcentration was variable (Fig.21). Evidence of septicaemia was also observed at post mortem examination and the experimental strain was isolated from every calf (Appendix II).

The Effect of IgG Treatment.

5 calves were given doses of IgG similar to, and multiples of, that present in the E.D.95 of whey (Fig.20). As far as clinical signs were concerned these calves most closely resembled the untreated control group in that they developed septicaemia within a short period after challenge. However, although survival time was similar in both groups, the onset of septicaemia was slightly delayed in IgG treated calves ($p < 0{\cdot}048$). Again there was considerable individual variation in haemoconcentration (Fig.22). The challenge organism was isolated from the peripheral blood and organs in all cases (Appendix II).

DISCUSSION.

The failure of IgG to influence to any extent the course of the disease is in accord with the inability, in vitro, to detect more than trace amounts of antibody to any E.coli antigens in this class of immunoglobulin by the method employed.

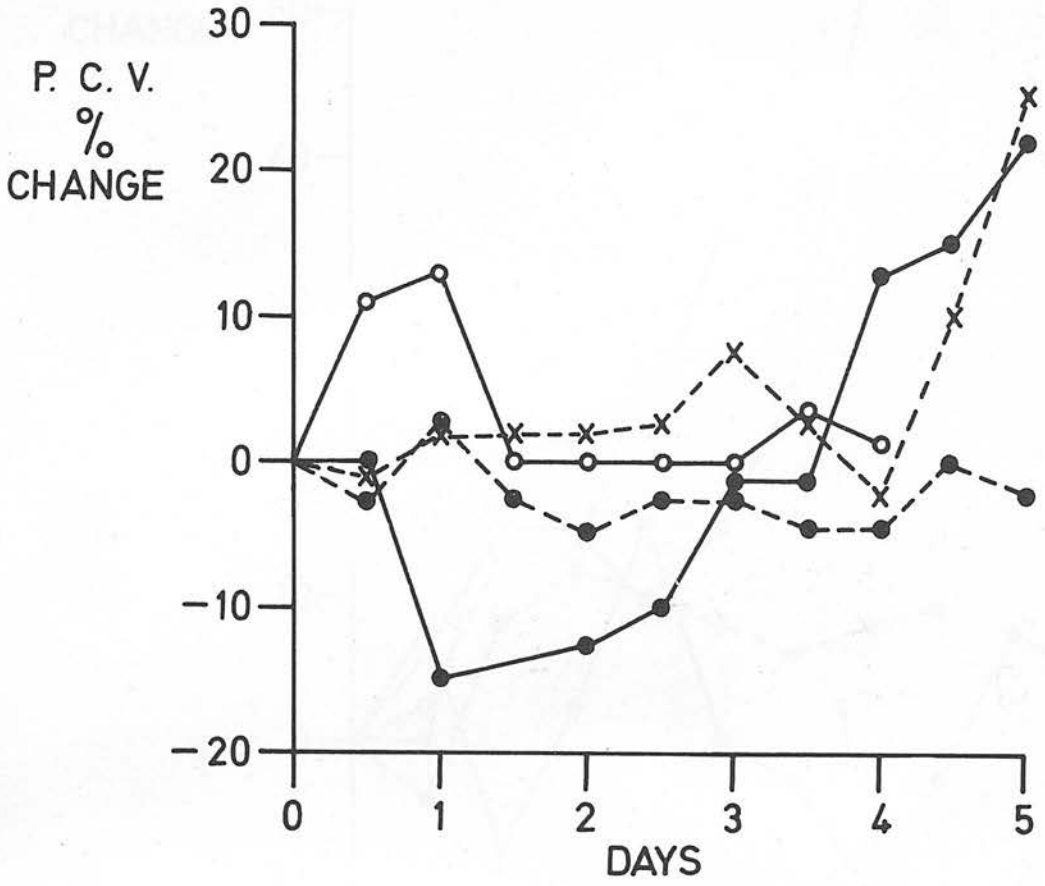

FIGURE 21

Daily changes in the packed cell volume of calves receiving IgM.

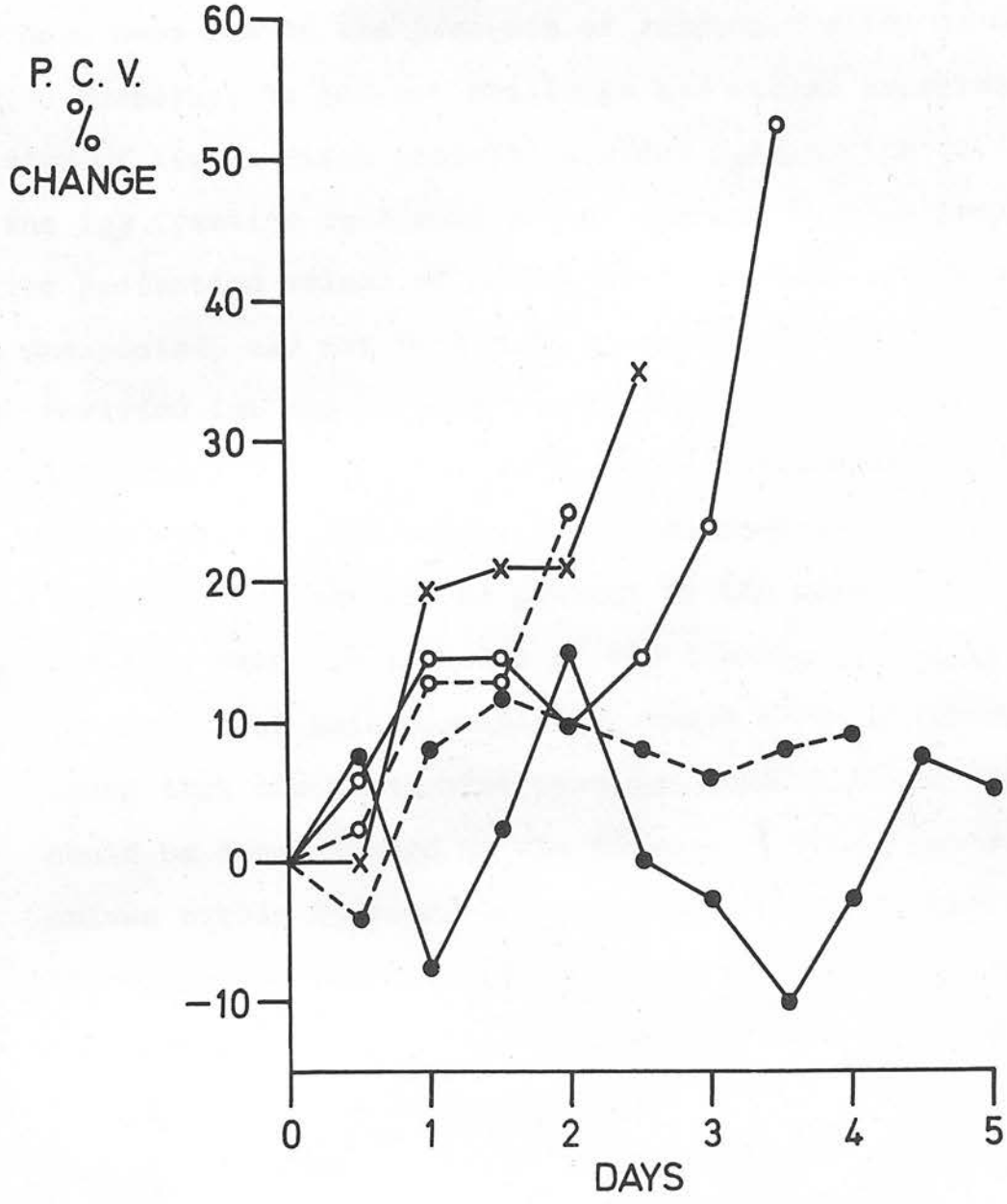

FIGURE 22

Daily changes in the packed cell volume of IgG treated calves.

This result does not support the opinion of Watt (1967) but since he was using only a crude gammaglobulin preparation, it is very likely that the prophylactic effect of his fraction may have been due to the presence of immunoglobulins other than IgG. Moreover, he did not challenge his calves experimentally. In view of its in vitro activity against E.coli, the failure of the IgM fraction in concentration similar to that contained in the protective volume of colostrum to prevent septicaemia was unexpected, and may be due to a number of factors:

(1) Purified IgM may be more slowly absorbed from the peritoneum than the IgM in natural whey, which may contain factors which enhance absorption. As a consequence, adequate levels of IgM may not be present in the circulation at the time when the invasion of the tissues by E.coli occurs. In support of this possibility, Smith & Halls (1968b) have shown that the particular serotype used in these experiments could be demonstrated in the tissues of colostrum-deprived calves within 2½ hours of challenge. Once in the tissues the organisms may be protected from the effect of IgM antibodies and may later re-enter the circulation when the IgM level has fallen.

(2) During preparation, partial denaturation of the IgM component may occur, even though biological activity appeared to be retained as judged by serological tests. This could lead to a more rapid clearance than that which occurs with untreated IgM in the whey.

(3) Antibodies to E.coli in different immunoglobulin classes may have a combined activity which is absent from the relatively pure fractions. It is also possible that antibodies in immunoglobulin classes other than those investigated here may be responsible for protection. In this connection IgA has definitely been identified in bovine colostrum by Mach, Pahud and Isliker (1969) and this finding has been confirmed in this and other laboratories. However, present day concepts ascribe a local protective function at the paramucosal surface of this class of immunoglobulin rather than systemic activity.

(4) A further possibility, although unlikely, is that colostral whey may contain protective factors in addition to immunoglobulins.

CHAPTER IV.

Whilst colostral IgM did not prevent colisepticaemia it nevertheless increased the resistance of the treated calves and markedly altered the pattern of the disease as seen in control calves. Clearly, it plays a major part in the prophylaxis of the calf against septicaemia. In order to investigate the role of IgM further, it was necessary to find an alternative source because colostrum was difficult to obtain in quantity and was invariably heavily contaminated with many types of bacteria. Colostral IgM is derived unchanged from the plasma and as bovine blood is readily available, in virtually unlimited quantities from abattoirs, it was considered as an alternative source. Abattoir blood has the further advantage that cattle slaughtered are drawn from a very large area and so should have a wide range of antibody activity against many serotypes of E.coli.

The present chapter describes a relatively simple procedure for obtaining an IgM-enriched fraction from normal bovine blood.

MATERIALS & METHODS.

Preparation of IgM-rich fraction.

The method employed for the concentration of IgM from serum is summarised in Fig.23.

Pooled bovine blood was collected at the abattoir as aseptically as practicable. When an animal had been stunned and elevated, the initial rush of blood after "sticking" was

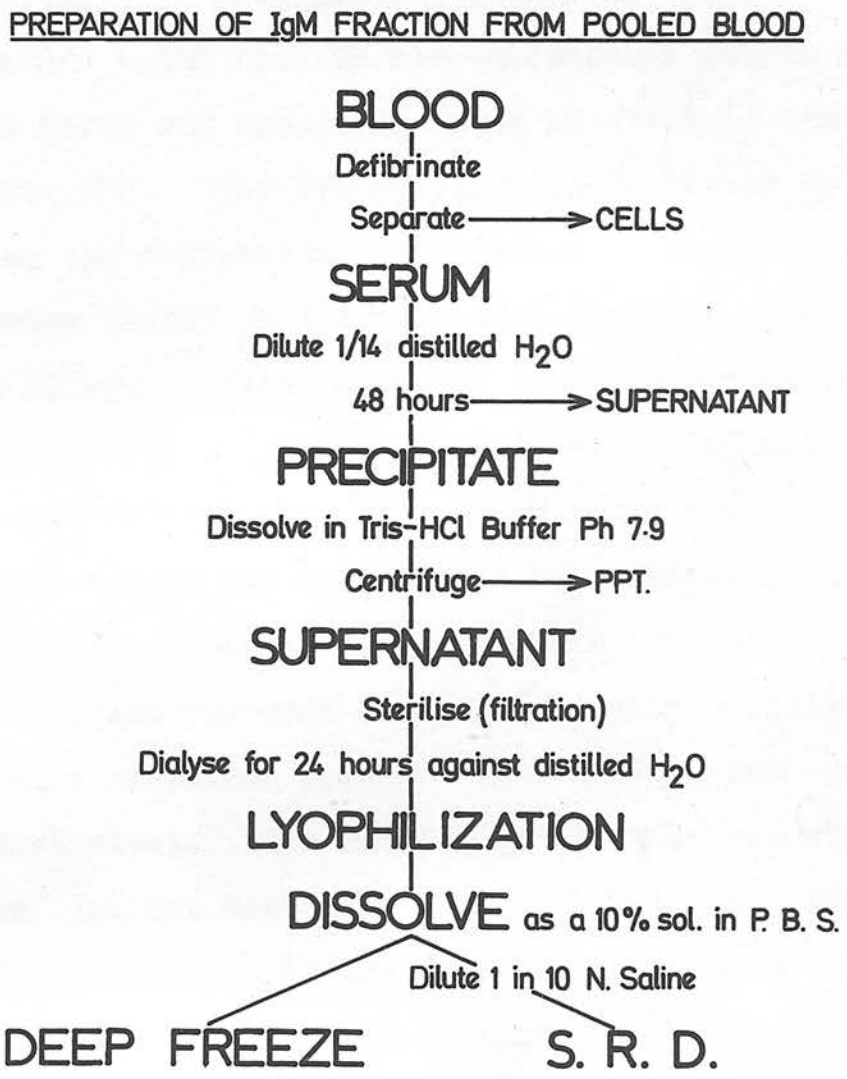

FIGURE 23

Flow diagram showing preparation of IgM-rich fraction.

collected in 10 litre plastic bottles, (Fig.24). The blood was defibrinated by hand stirring and bulked in 25 litre aspirators (Fig.24). It was then taken to the laboratory where the rest of the fractionation was completed. The blood was filtered through a coarse nylon mesh to remove residual fibrin clots and then the serum was recovered using an electric cream separator (Fig.25). The IgM fraction was obtained by precipitating the euglobulins by dilution with deionised distilled water (1:14) at 4°C in large polythene tanks each holding 150 litres. After 48 hours the precipitate was harvested by centrifugation in a continuous flow rotor (M.S.E.) (Fig.26) and redissolved in 0·1M tris HCl buffer (pH8) containing 1M NaCl using 20 volumes for every litre of original serum. Insoluble matter was removed by centrifugation for 1 hour and the supernatant was rendered bacteriologically sterile by filtration on a cellulose membrane with 0·22 μ average pore diameter (Sartorius). The sterile supernatant was dialysed for 24 hours, against distilled water (50 volumes), and lyophilised. Finally, the fraction was resuspended as a 10% solution in phosphate buffered saline (pH 7·8) and stored at -25°C. Samples from different batches were assayed serologically and immunochemically.

Immunoelectrophoresis, quantitative immunoglobulin determination, total protein estimation and indirect haemagglutination tests were carried out as reported in Chapter I.

FIGURE 24

A 10 litre bottle.

B 25 litre aspirator.

FIGURE 25

Electric Cream Separator.

FIGURE 26

Continuous Flow Rotor.

Gel Filtration Chromatography.

Adult serum and the IgM fractions were fractionated on a 60 x 4·4 cm. column of G 200 Sephadex (Pharmacia Ltd.) using 0·1M tris HCl buffer (pH 8·0) containing 1M NaCl.

Electrophoresis.

Conventional electrophoresis was also carried out on cellulose polyacetate strips (Sepraphore III, Gelman Instrument Co.) with 0·05M sodium barbitol-barituric acid tris buffer (pH 8·8). The strips were stained with Ponceau S and scanned on a densitometer (Kipp & Zonen).

RESULTS.

Using the above technique, it was possible to fractionate up to 100 litres of blood weekly.

Analysis of IgM fractions.

Successive batches of the IgM rich fraction prepared by precipitation with distilled water were compared with their respective parent serum pools by a number of analytical methods.

Fig.27 shows the typical profile of this IgM rich fraction obtained by chromatographic separation on a column of G 200 Sephadex. Of the two peaks which are apparent, the first, composed of large molecular 19S components including IgM, is larger than the second 7S peak. This contrasts with the elution profile of serum on the same material where the 19S first peak is smaller than the second 7S peak and where an additional third 4·5S albumin-containing peak is observed. Analysis by electrophoresis on cellulose polyacetate membrane

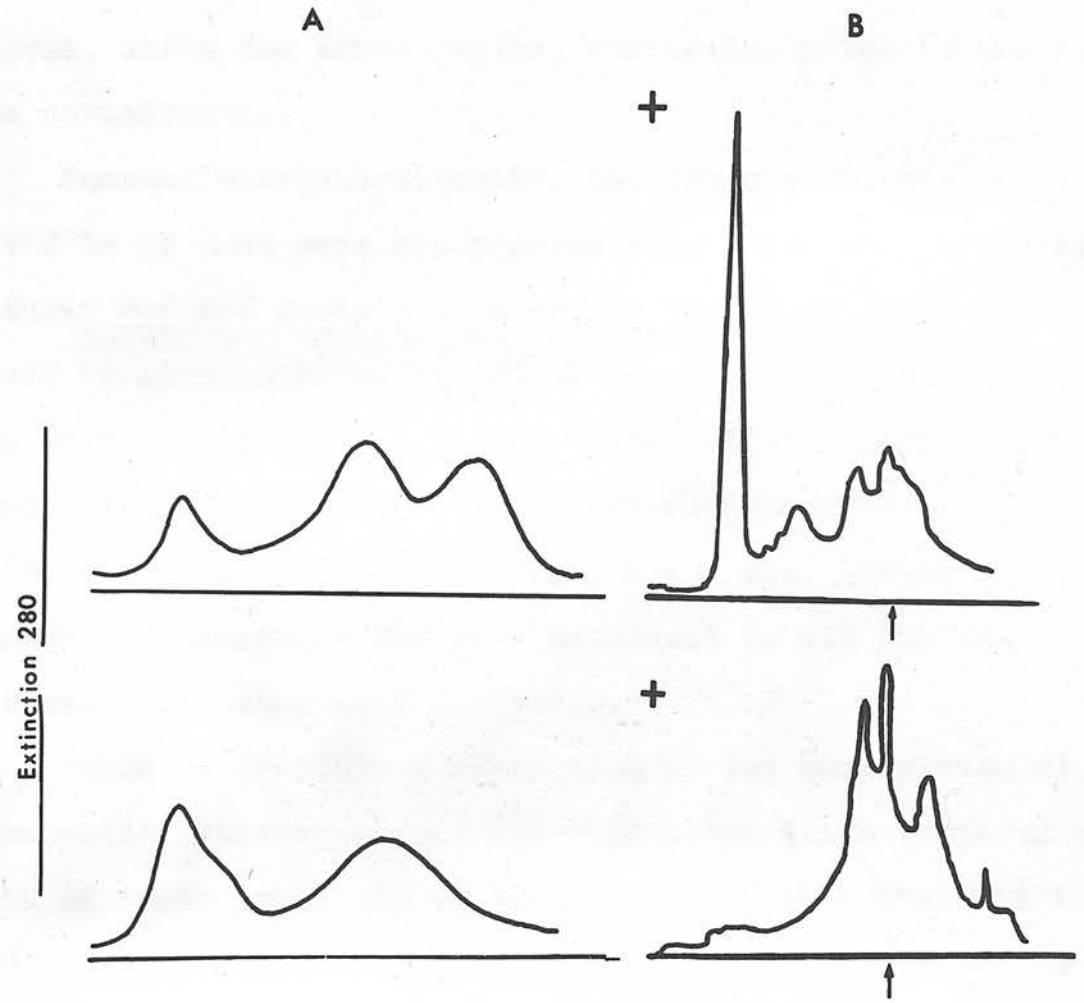

FIGURE 27

A Sephadex G 200 chromatograms of pooled adult serum (upper) and IgM-rich fraction (lower);

B Electrophoretic profile of pooled adult serum (upper) and IgM-rich fraction (lower). Arrow - point of sample application.

Fig.27 revealed that the IgM preparation has a similarly restricted protein profile by this method as compared to the parent serum sample. In this case the electrophoretically fast albumin peak and β components were almost entirely absent, while the gamma region, particularly the faster portion, was concentrated.

Immunoelectrophoretically, the IgM preparation was also found to be much more homogeneous than serum and only five clearly defined protein arcs mainly in the β and γ regions could be readily observed (Fig.28). Furthermore, the IgM arc was more prominent than in serum and albumin barely apparent, indicating a reversal of the concentrations found in the parent material. In addition to these, a $\gamma 1$ arc, thought to be a complement component was very prominent in all the IgM preparations using this technique.

Table VI presents a comparison of the composition of five consecutive batches of the IgM rich preparation compared with that of their parent serum pools. This shows that for an equivalent protein content a substantial concentration of IgM was consistently obtained and this component, invariably the predominant molecular species, represented approximately half the total protein present. In contrast, IgG was not concentrated by this procedure and was present at about the same concentration as in serum. Antibody titre to the somatic antigen (O) of E.coli serotype O9K(A?)was increased to a level corresponding to the degree of IgM concentration. However, the yield of

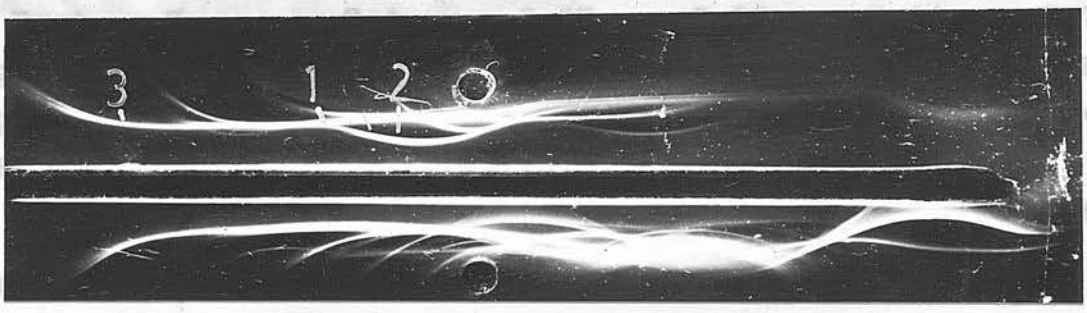

FIGURE 28

Immunoelectrophoresis of IgM-rich fraction (upper well) and pooled adult bovine serum (lower well) against rabbit anti-whole bovine serum (trough)

1 IgM
2 7S γ1
3 IgG

IgM recovered in each batch represented only a small proportion (approximately 5%) of the total IgM present in the starting volume of parent serum pool used.

Table VI.

Analyses of Successive Batches of IgM-Rich Fraction Compared with Parent Serum Pools.

	1	2	3	4	5
Serum					
Volume, litres	10	20	10	10	10
Total Prot. g.%	8.4	6.57	7.68	7.44	5.7
IgM mg. per ml.	5.9	3.9	4.7	4.8	3.05
IgG mg. per ml.	25.5	15.1	16.65	14.0	14.4
O9 Titre	128	128	128	128	64
Total IgM g.	59.0	78.0	47.0	48.0	30.0
IgM Prep. 10% Sol.					
Volume, ml.	55.0	87.5	101.0	55.5	59.0
Total Prot. g.%	7.98	8.07	7.77	8.34	8.64
IgM mg. per ml.	49.0	37.0	42.0	43.0	50.0
IgG mg. per ml.	15.0	15.0	10.0	10.0	13.0
O9 Titre	4,096	2,048	2,048	4,096	2,048
Total IgM g.	2.69	3.23	4.24	2.38	2.95

DISCUSSION.

The method of preparation described, offered a simple procedure for obtaining a suitable quantity of IgM in a convenient volume, thus permitting amounts approximating to that received by the calf under natural conditions by the ingestion of colostrum, to be given by a single injection. At the

concentration of IgM which was achieved (circa 35 - 40 mg./ml.) it was found that the antibody titre against the selected O antigen used in the serological test was similar to that which could have been obtained by hyperimmunisation (Penhale, 1965). Whilst this preparation was not pure and contained other serum proteins, it was considered suitable to use to investigate IgM activity in relation to colisepticaemia, as the only other immunoglobulin present was IgG which has been shown to have little or no effect on the syndrome.

CHAPTER V.

In this chapter, the effect of the IgM fraction on coli-septicaemia was examined. Whilst successive batches appeared to have similar IgM content and antibody activity, it was considered advisable to prepare a large pool of standard material prior to use and so avoid any possibility of individual variation. The IgM-rich fraction was administered intra-peritoneally in order that a direct comparison could be made with the results in previous chapters.

MATERIALS & METHODS.

Preparation of the Pooled Serum IgM rich Fraction.

Batches of the serum IgM fraction were prepared weekly and stored freeze dried. When sufficient IgM had been collected to pretreat 30 - 40 calves, the freeze dried material was pooled and dissolved in phosphate buffered saline as before. It was stored at - 20°C in aliquots of 30 ml. and a sample retained for immunochemical analysis.

Calves.

Newborn colostrum deprived calves were managed as before. The calves were given intraperitoneally varying doses of the IgM fraction containing IgM in excess of that in the colostral whey, and infected 2 hours later with the experimental *E.coli* serotype O78K80(B). 7 control calves were given only the *E.coli* culture. The results were examined statistically by the randomisation test.

RESULTS.

The analysis of the pooled IgM fraction is summarised in Table VII. The composition of the fraction was similar to that of earlier preparations and contained 37 mg./ml. of IgM and 10 mg./ml. IgG. The calves tolerated the IgM fraction quite well when it was given intraperitoneally but, as with the whey fractions, there was some depression. This generally passed within 1 - 2 hours and was thought to be due to the presence of toxic factors in the serum fraction. As with the whey, it is likely that these factors resulted from bacterial contamination during collection of blood.

Table VII.

Analysis of Pooled IgM-Rich Fraction (10%) solution w/v.)

Total Protein g.%	IgM mg./ml.	IgG mg./ml.	Haemagglutination Titre 09	Haemagglutination Titre 078
8·10	37	10	2048	1024

The IgM fraction was administered prophylactically to 29 newborn colostrum deprived calves which were subsequently infected with the experimental serotype. The results are summarised in Fig.29 where it can be seen that, as with the parenteral administration of colostral whey, the IgM fraction failed to any great extent to influence diarrhoea. In 12 calves, septicaemia was excluded but 6 of these died with an acute diarrhoea. It was found that even very small quantities

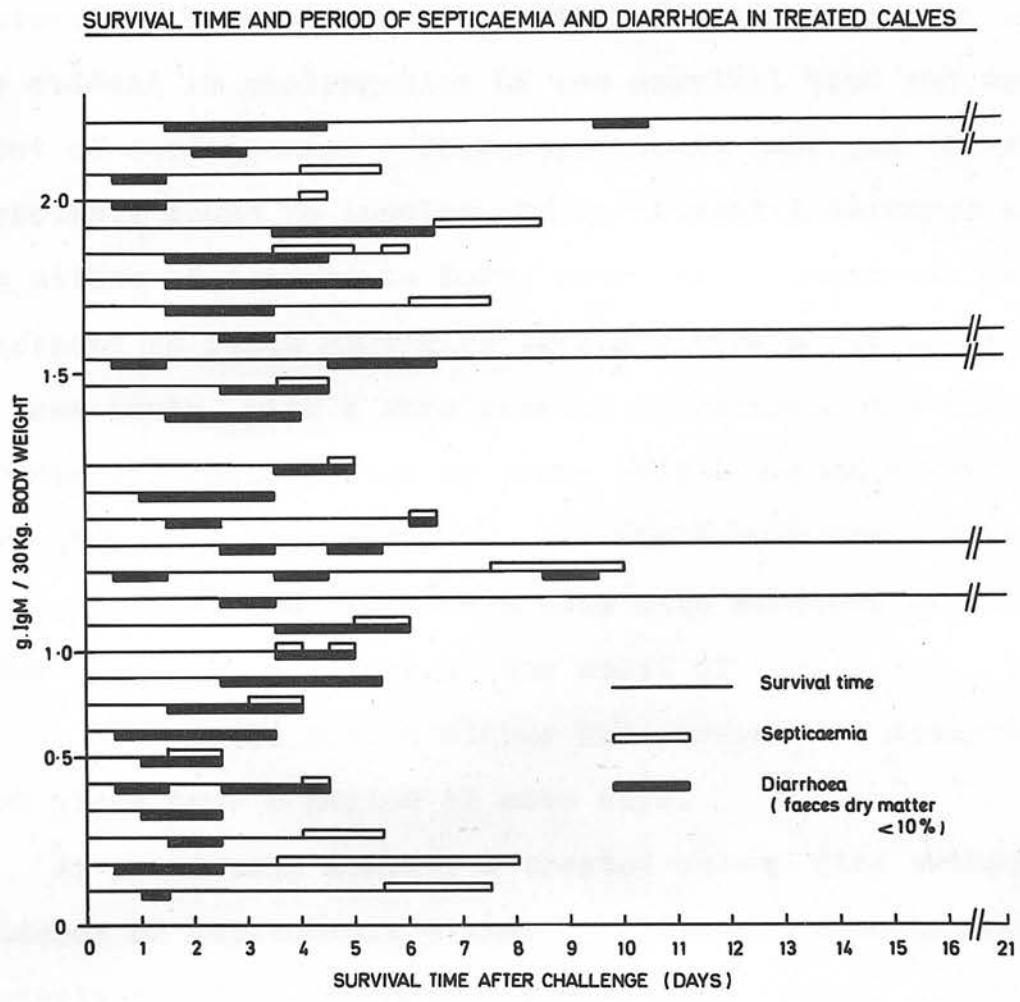

FIGURE 29

Survival time and periods of septicaemia and diarrhoea in pre-treated calves.

of this preparation had a marked influence on the usual course of the experimental disease as observed in untreated control calves (Fig. 30). Although complete protection was not achieved with these low doses, the effect of the fraction was evident in prolongation of the survival time and delayed onset of septicaemia. When septicaemia occurred it was invariably found to involve the experimental serotype and was either of a peracute form, where the bacteraemia rapidly increased to reach very high levels within a few hours of onset, or less acute, with a more gradual increase in the numbers of circulating bacteria taking place over a period of several days (Fig. 29, Appendix III). In the former type, deterioration was very rapid and the calves died with symptoms of extreme shock within a few hours of the onset of bacteraemia, while in the less acute form a slower but progressive deterioration took place over a period of some days.

As previously stated, 6 treated calves died without evidence of septicaemia either by ante-mortem isolation of bacteria from the peripheral circulation or from the tissues post mortem and in these cases the most prominent clinical feature was severe diarrhoea (Fig. 29). Although the experimental serotype was rarely isolated from the gastro-intestinal tract, in the majority of these calves mucoid strains of E.coli could be cultured from the upper intestine and in some cases also from the mesenteric lymph nodes (Appendix III).

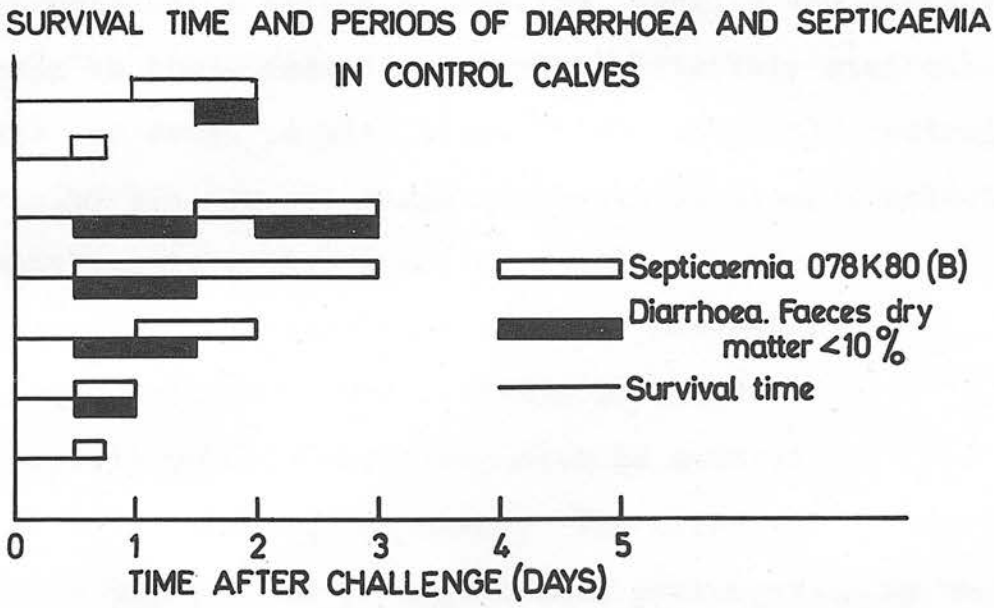

FIGURE 30

Survival time and periods of diarrhoea and septicaemia in control calves.

When given at levels of over 1 gm. (approximately 30 ml. of IgM fraction) a number of calves survived and showed no evidence of septicaemia during the experimental period. Survival occurred irregularly and appeared to be unrelated to dosage above the 1 gm. level.

The prophylactic effect of the IgM fraction was clearly evident even in those individuals which ultimately died when the results are compared with those of the untreated control calves (Fig.30). In all cases untreated challenged calves died of septicaemia within a period of 72 hours from the time of infection (mean survival time 43 hrs.) and onset of bacteraemia was always observed within 36 hours.

The overall results were subjected to statistical analysis by the randomisation test. The effect of prophylactic IgM treatment was evident in significant prolongation of the survival time of all calves which died, whether of the septicaemia/scour complex or enteric disease alone ($p < 0{\cdot}002$). It was also found that in the calves which ultimately developed septicaemia, pretreatment with IgM fraction before challenge, irrespective of dosage level, significantly prolonged the interval before onset of bacteraemia ($p < 0{\cdot}001$). Furthermore, the preparation also appeared to have some minor influence on the enteric situation as pretreatment significantly prolonged the interval before onset of diarrhoea ($p < 0{\cdot}005$).

DISCUSSION.

The failure of the preparation consistently to afford complete protection despite its biological activity was not unexpected in the light of the previous experiments where more purified colostral IgM was used. It is possible that several factors may have contributed to this failure. Firstly, the toxicity of the fraction, which was thought to be due to the presence of contaminating bacterial products, may have adversely affected the ability of the calf to withstand challenge with the highly virulent strain of E.coli used. In this context it is well established that endotoxins, particularly of gram negative bacteria, can markedly reduce the resistance of animals to infection (Wright, 1904; Condie, Zak & Good, 1955; Dubos & Schaedler, 1966; Conti, LeCluff & Epscheder, 1961). These authors demonstrated that pretreatment with endotoxins of E.coli and Shigella flexneri reduced the number of organisms necessary to kill mice and rabbits. Secondly, as suggested in Chapter II, it is possible that the severe diarrhoea which developed in nearly every calf could have an influence on the level and duration of the systemic immunity provided by the injection of IgM fraction. Scouring reduces the half life of IgG considerably (McDougal & Mulligan, 1968) and by analogy with the estimation of the half life of human IgM (Barth, Wochner, Waldmann & Fahey, 1964) it is possible that the normal half life of bovine IgM is much

shorter than that of bovine IgG and therefore, more likely to be severely affected by this factor. Though it has been established that IgM is absorbed from the peritoneum it is probable that there was considerable individual variation in the percentage and rate of absorption by each calf.

The predominance of diarrhoea despite the administration of large amounts of parenteral immunoglobulin supports the hypothesis based on the earlier studies (Chapter II) that systemic IgM only provides partial immunological cover which appears to be largely limited to the vascular compartment. However, the present studies revealed a small but significant influence of IgM fraction within the gastro-intestinal tract in that this preparation delayed the appearance of enteric disease.

CHAPTER VI

In the earlier sections, it has been postulated that various factors may have contributed to the failure of IgM fully to protect calves against septicaemia.

(1) IgM may be poorly and irregularly absorbed from the peritoneum and thus, when the calves were challenged with E.coli, there would be inadequate serum levels to prevent invasion of the tissues by the organism. In addition, there could be considerable variation in absorption in individual calves.

(2) Partial biological denaturation of IgM may occur during preparation.

(3) Diarrhoea, may cause increased catabolism of immunoglobulin.

(4) Toxic contamination of the preparations could adversely affect the calves' natural resistance to infection. At the higher dosage levels, the toxic effect could predominate over the prophylactic value of the IgM. Because both the colostrum and abattoir blood were found to be heavily contaminated with E.coli it was thought likely that endotoxin could be the principal toxic factor in the preparations previously used. In this respect, it has been demonstrated that neonatal calves are particularly susceptible to endotoxin shock (Penhale, 1965).

To examine the above hypothesis it was necessary to prepare non-toxic IgM which could be administered intravenously. To this end, a preliminary examination of the toxicity was

carried out.

MATERIALS & METHODS.

Two IgM fractions, one prepared aseptically from blood taken from living cows and the other prepared from abattoir blood, were respectively injected intravenously into 2 newborn colostrum deprived calves and the results compared.

Preparation of IgM Fraction from Living Cows.

Living animals were chosen because it is relatively simple, using aseptic techniques, to collect blood free from any bacterial contamination.

5 Ayrshire cows were used as donors. An area of skin over the external jugular vein was shaved and disinfected with ethanol. 6 litres of blood from each cow was collected from the vein, using a 14 gauge needle attached to sterile vinyl tubing, into sterile evacuated M.R.C. blood bottles which contained some glass beads. Immediately, upon collection the bottles were shaken gently to defibrinate the blood; the fibrin adhering to the beads. The serum was removed by centrifugation of the bottles, pooled, and the IgM fraction prepared as previously described. At each stage of fractionation, bacteriological examination was carried out.

Preparation of IgM Fraction from Abattoir Blood.

20 litres of blood were collected from 2 bullocks. After stunning, when the animals had been raised, a longitudinal incision was made through the skin of the ventral surface of the neck. The skin was reflected back prior to bleeding, so

that blood did not come into contact with the hair of the animals. The blood was collected in 2 sterile containers, defibrinated and fractionated as before. Again, samples were checked at every stage for bacterial contamination.

Bacterial Examination.

Throughout the IgM preparation, samples were taken for bacteriological examination. ·25 ml. of each sample was spread directly on the surface of McConkey and sheep blood agar plates which were incubated overnight.

Blood Examination.

Endotoxins cause a very pronounced leucopenia; leucocytes disappearing rapidly from the circulation, followed by lymphocytes (Braude, 1964; Penhale, 1965). Therefore a drop in white cell count can be used as a measure of the toxicity of the preparations. White cell counts were carried out using an improved Neubauer haemocytometer slide.

RESULTS.

The IgM prepared from living donors was sterile throughout the process but the initial sample from the abattoir contained 1 colony of E.coli per plate. The results of intravenous administration of the appropriate preparation at a dosage of 40 ml./30 kg. body weight to 2 calves are summarised in tables VIII and IX.

TABLE VIII

The Effect of Administering Intravenously an IgM-Rich Serum Fraction Prepared Aseptically from Blood taken from Living Donors.

Calf weight 27 kg. - dose given 35 ml. 10% w/v Solution.

Time after inoculation	Temperature °F.	W.B.C./ cu.mm.	Clinical Observations
0	100·8°	7,000	35 mls. were given intravenously over 1 minute. After the injection, calf lay down in normal position. It was bright and interested in surroundings and when encouraged was able to rise.
10 min.	100·8°	No sample	Calf fed 3 pints milk. Very hungry.
30 "	100·9°	4,500	Calf bright and normal.
60 "	100·6°	4,500	Calf bright and normal.
90 "	100·4°	4,500	Calf sleeping, bright when disturbed.
120 "	100·8°	4,500	Calf sleeping, bright when disturbed.
3 hrs.	100·8°	4,500	Calf sleeping, bright when disturbed.
4 "	101·0°	5,000	Calf lying, normal.
5 "	101·8°	*5,000	Calf lying, normal.
5½ "	101·4°	*6,000	Calf bright and hungry. Fed 3 pints of milk.
24 "	101·6°	9,250	Calf bright and hungry. Fed 3 pints of milk.

* These 2 samples were stored over night, whilst the others were measured immediately after sampling.

TABLE IX

The Effect of Administering Intravenously an IgM-Rich Serum Fraction Prepared from Slaughterhouse Blood.

Calf weight 39 kg. - dose given 50 ml. 10% w/v Solution.

Time after inoculation	Temperature °F.	W.B.C./ cu.mm.	Clinical Observations
0	102°	7,000	After receiving 20 mls. the calf sank to the ground and lay flat out. In less than 2 minutes it recovered. 30 ml. was then given slowly over 1 minute and the calf remained standing. It was willing to suckle and took 3 pints of milk.
30 min.	101·1°	5,000	Calf standing.
60 "	101·6°	4,000	Calf standing.
90 "	101·0°	2,000	Calf lying, when raised it was able to stand.
120 "	101·4°	1,000	Calf lying, bright.
150 "	101·6°	1,000	Calf lying, bright.
3½ hrs.	102·3°	1,000	Calf lying, bright.
4½ "	101·8°	2,000	Calf lying, bright.
5½ "	101·6°	2,000	Calf bright, drank 3 pints of milk.
6½ "	101·0°	1,500	Calf bright.
24 "	102·3°	5,000	Calf bright and hungry, 4 pints of milk taken.
31 "	101·3°	6,000	Calf bright and hungry, 4 pints of milk taken.

The IgM prepared from living donors caused no clinical signs but the abattoir fraction produced slight hyperpnea and muscular weakness from which the calf quickly recovered. Both calves fed greedily within 10 minutes after injection of the fractions. Neither calf had a significant rise in temperature but a drop in total white cell count was observed in both calves. The white cell count reached a minimum 2 hours post-injection and then gradually rose to approximately pre-injection levels within 24 hours.

DISCUSSION.

This study, although limited, nevertheless was sufficient to indicate that it was possible to prepare fractions which were well tolerated by calves, even when given intravenously. The results suggested that the toxicity was not inherent in preparations per se and so the toxicity of earlier preparations could be attributed to bacterial contamination. As *E.coli* was the major contaminant found, it is likely that endotoxins are the chief cause of this toxicity and this is supported by the observation, that the abattoir preparation (from which *E.coli* were isolated) had a greater depressant effect on white cell count than the fraction prepared from the living donors.

CHAPTER VII.

The ability to prepare a relatively innocuous IgM fraction from abattoir blood made it possible to examine the different factors which might have influenced the activity of the various preparations used in the earlier studies. Despite the use of different preparations at various dosages, it was interesting to note that the survival time and delay in onset of septicaemia in different groups was very similar (Table X).

TABLE X

Survival Time and Delay in Onset of Septicaemia of Pretreated Septicaemic Calves.

Group of Calves	Survival Time (days)		Delay in Onset of Septicaemia (days)	
	Mean	Range	Mean	Range
Whey Treated	5·4	2·5 - 16·0	4·8	1·5 - 15·0
Colostral IgM	4·8	4·0 - 5·0	4·0	3·5 - 4·5
Serum IgM fraction	6·0	2·5 - 10·0	4·5	1·5 - 7·5

This would suggest that some factor or factors were common to all 3 preparations. It was considered that IgM could be the common link. In other species, it has been established that the half life of IgM is quite short. Barth, Wochner, Waldmann & Fahey (1964) reported that human IgM had a half life of 4 - 6 days, whilst Porter & Hill (1970) stated that the half life of porcine IgM was even shorter (1 - 3 days). If bovine IgM has a similarly short half life, then the IgM passively acquired by the calf from the various preparations administered, would be quickly catabolised. As a consequence of the fall in serum

IgM levels the calf would be susceptible within a short time to reinfection from its environment.

To examine this hypothesis together with the others formulated in the preceding chapter, calves were given serum IgM intravenously in single and double doses. At the same time, immunological and serological examinations of the calves' sera were carried out in order to investigate the daily changes in the immune status of the calves.

MATERIALS & METHODS.

Serum IgM Preparation.

Bulk quantities of the IgM fraction were prepared from abattoir blood but with the modification that all pooled blood was checked for sterility and only samples with minimal bacterial contamination (not more than 8 colonies/ml.) were used. Similar checks were also carried out at strategic points during the fractionation process (Appendix IV).

Calves.

Newborn colostrum-deprived Ayrshire bull calves, obtained from a closed herd, were managed as previously described (Chapter II). 6 calves were given a single intravenous injection of the IgM rich serum fraction containing a standard dose of 1 gm. IgM/30 kg. bodyweight and 2 hours later, they were infected orally with the experimental E.coli 078K80(B) serotype. 12 calves were similarly treated, but in addition were given a further dose of the preparation intravenously on the fourth day post-challenge. This particular time interval was chosen

because it was found that by previous experience, the onset of septicaemia was delayed approximately 4 days when a single dose was administered intraperitoneally (Table X). To avoid the possibility of favourable seasonal conditions 7 calves were treated during the late summer and early autumn (August - October) and 5 were treated during mid-winter (December - February). During the course of the experiment the virulence of the E.coli organism was checked by regularly challenging untreated control calves. Calves were kept under observation over a period of 14 days during which time blood samples were taken twice daily for bacteriological and serological examination.

Quantitative Immunoglobulin Determination.

The daily serum levels of immunoglobulins were measured quantitatively by the single radial diffusion technique.

Indirect Haemagglutination and Antiglobulin Tests.

Chicken erythrocytes were sensitised with an O antigen extract of the challenge E.coli serotype O78K80(B) as described in Chapter II. The daily serum samples were inactivated by heating at 56°C for 30 minutes. In the titration of antibody a 1% suspension of the sensitised erythrocytes was added to serial two-fold dilutions of serum. Each sample was tested for natural haemagglutinins by incubating with unsensitised cells. For the tests, the method of Buxton (1959) was followed except that 0·2 ml. volumes were used.

RESULTS.

The serum IgM fraction, when prepared from sterile blood,

was found generally to be well tolerated when administered by the intravenous route. In some cases, following injection, no observable reaction occurred but, more frequently, slight depression and hyperpnea were noted immediately post-injection, and occasionally this was followed by muscular weakness and recumbency. In all cases where a reaction was seen this was of short duration and calves invariably recovered within 5 minutes.

Prophylactic Studies.

The prophylactic effects of this preparation in the experimentally infected calves are summarised in Figs. 31 and 32. The 6 calves which received one dose of IgM fraction, died of septicaemia. The experimental serotype was recovered from the peripheral blood in 5 calves and from the organs post-mortem in every case (Appendix V). The mean survival time (6 days post-challenge) and delay in onset of septicaemia (5 days) was comparable to that seen in calves receiving colostral whey, colostral IgM or serum IgM intraperitoneally (Table X).

In contrast, none of the calves receiving two doses of the serum IgM fraction became bacteraemic, except on one occasion when 20 colonies/ml. were isolated from the blood of one calf on the ninth day (Fig.32 and Appendix V). This calf, at that time, appeared to be clinically normal and it may be significant that it was penned adjacent to one which had been infected 24 hours previously. Of the 12 calves, 10 survived, while one in each group died of enteric disease.

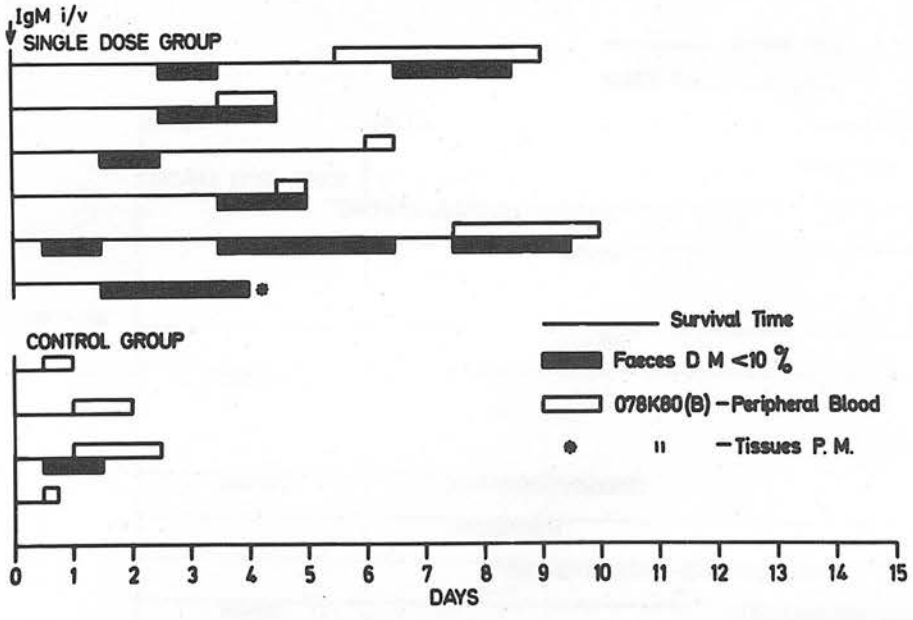

FIGURE 31

Survival time and periods of diarrhoea and septicaemia in control calves and calves receiving one dose of serum IgM fraction.

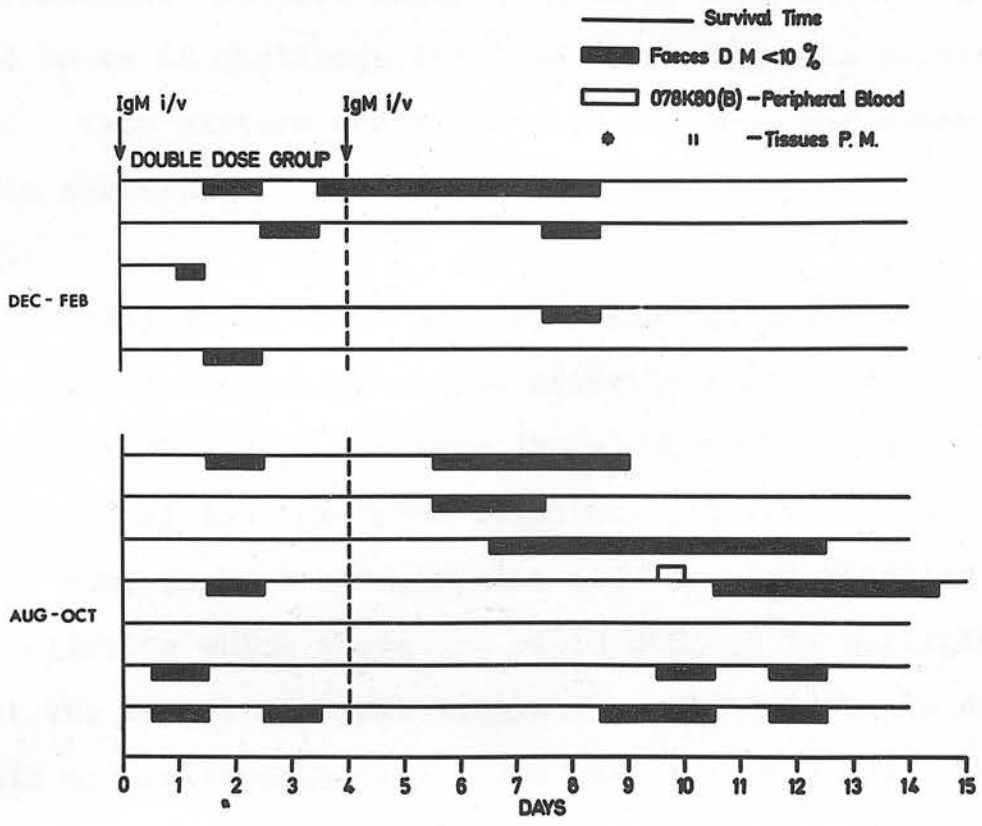

FIGURE 32

Survival time and periods of diarrhoea and septicaemia in calves receiving two doses of serum IgM fraction.

Bacterial examination of these 2 calves post mortem failed to demonstrate the presence of E.coli in the tissues, but mucoid strains of E.coli were isolated from the small intestine and mesenteric lymph nodes. As before, diarrhoea was seen, apart from one exception, in all calves.

The untreated control calves (Fig. 31) became bacteraemic within 24 hours of challenge and died of septicaemia within 60 hours. This pattern conformed with the previous experience using this serotype.

Serology.

The pre-injection serum samples contained neither antiglobulin nor haemagglutination activity against the O antigen of the challenge serotype (Figs. 33 & 34). After administration of the IgM serum fraction, all calves showed an initial rise in both antiglobulin and haemagglutination titres, following which there was rapid decline to negligible levels at the fourth day post challenge. In the single dose group this was followed by a rise on the fifth day (Fig. 33).

In the case of those receiving the double dose (Fig. 34) a second peak of antibody activity corresponded with the time of injection of the second dose, after which antiglobulin and haemagglutination titres again declined until the seventh day when levels once more began to rise slowly.

The daily serum IgM levels followed a similar pattern to the haemagglutination titres. The IgG serum levels in both groups of calves increased gradually from birth, but

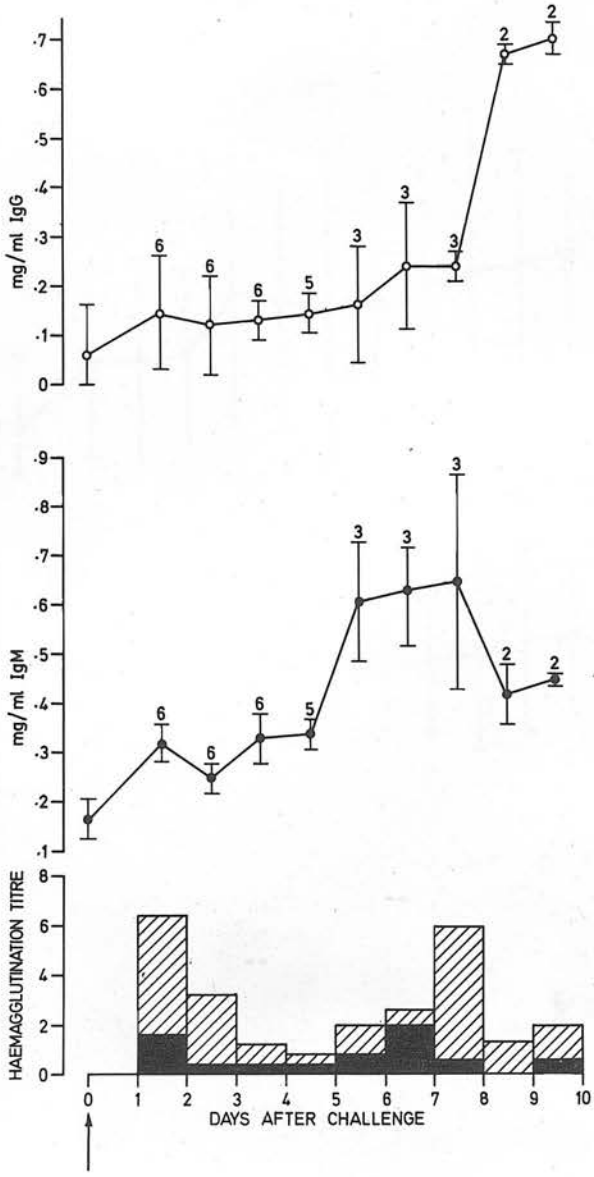

FIGURE 33

Plasma haemagglutination and antiglobulin titres, IgG and IgM levels with standard deviations, in calves receiving one dose of serum IgM fraction. Arrow indicates time of injection of IgM fraction; haemagglutination titre represents the arithmetical mean of the daily indirect haemagglutination titres (black) and antiglobulin titres (shaded) figures. Above S.D. are the numbers of calves sampled.

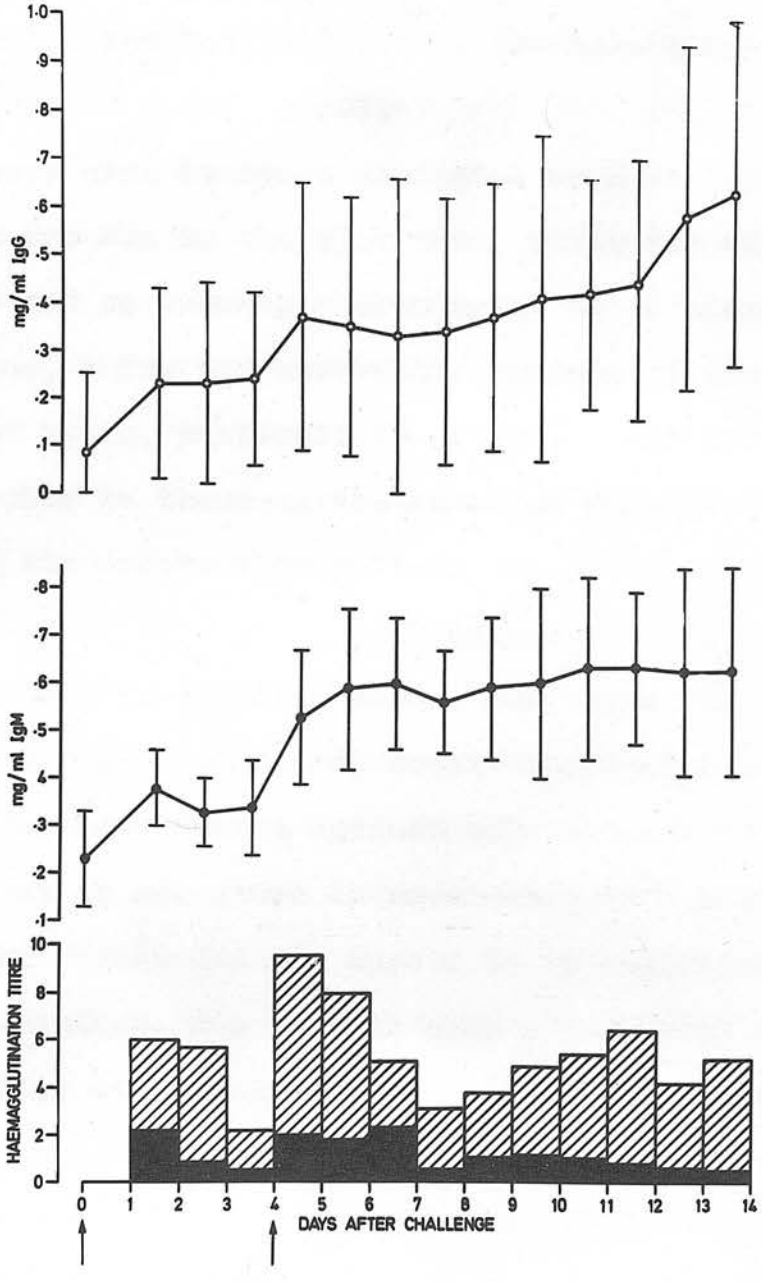

FIGURE 34

Plasma haemagglutination and antiglobulin titres, IgG and IgM levels with standard deviations, in calves receiving two doses of serum IgM fraction. Arrows indicate times of injection of IgM fraction; haemagglutination titre represents the arithmetical mean of the daily indirect haemagglutination titres (black) and antiglobulin titres (shaded).

throughout the observation period remained low relative to adult or colostrum fed calf levels (Penhale & Christie, 1969; Klaus, Bennett & Jones, 1969).

The very wide standard deviation seen in both IgG and IgM levels was due to the fact that, while the majority of the calves had no immunoglobulin prior to injection of the IgM fraction, a few had measurable amounts of foetal immunoglobulin at birth, particularly of IgG. The levels of immunoglobulin in these calves remained consistently higher throughout the observation period.

DISCUSSION.

These results indicate that a non-toxic serum IgM fraction, at the dosage level employed, could consistently protect colostrum-deprived calves against experimental colisepticaemia provided that it was given intravenously in 2 suitably spaced doses. The result did not appear to be influenced by any seasonal variation, one calf in each group, kept at different periods, dying of enteric disease. During this experiment, while the calves suffered from diarrhoea, it did not appear to be quite so severe as in earlier experiments, and it may be that the enterotoxigenic E.coli which spontaneously infected calves was not as virulent as in earlier studies. On the other hand, the earlier IgM preparations were known to be toxic and in consequence may have depressed the resistance of the calves or perhaps even actively precipitated the diarrhoea. In the latter context, it has been stated (Wray & Thomlinson, 1969)

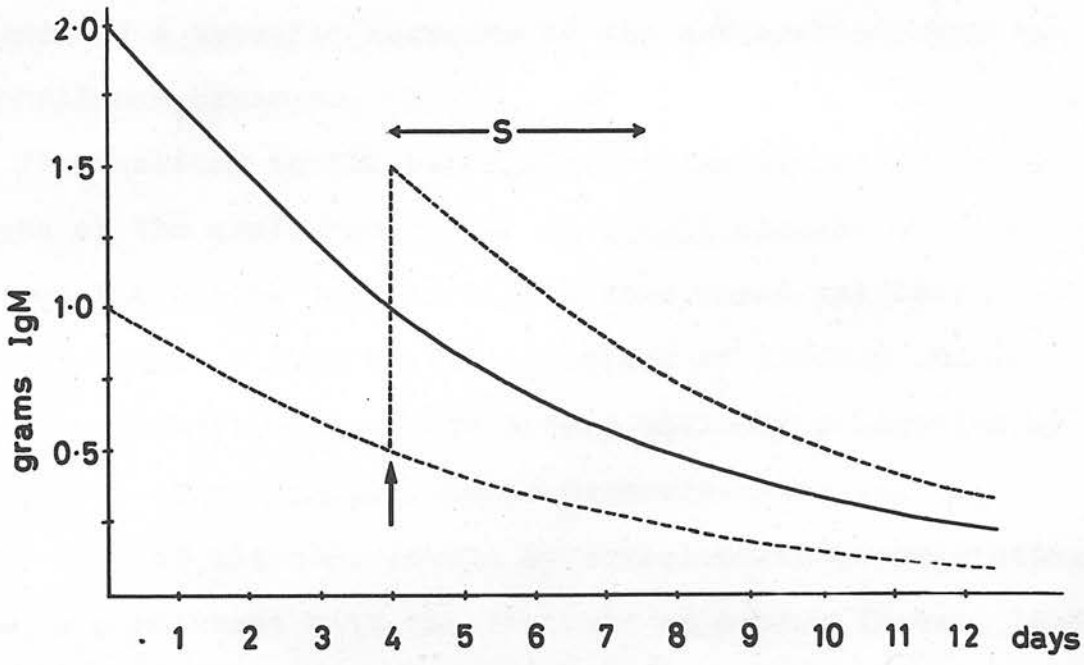

FIGURE 35

Theoretical decay curve of IgM having a half life of four days ← S → represents period when onset of septicaemia was observed in single dose calves. Arrow indicates time of second injection.

superimposed upon the sharp increases immediately following the administration of the IgM fraction. In contrast to IgG, IgM levels in calves which survived were approaching those of the adult at the end of the experimental period. In the single dose group, an abrupt rise in plasma IgM levels accompanied the onset of septicaemia suggesting that this represented a specific response to the antigens present in the challenge organism.

Fluctuations in the haemagglutination titres to the O antigen of the challenge strain of E.coli closely paralleled those of the plasma IgM indicating that these antibodies were mainly present in this particular class of immunoglobulin.

The evidence obtained of active antibody production by both quantitative immunoglobulin determinations and the measurement of antibody levels by antiglobulin haemagglutination tests is consistent with the findings of others (Brown, 1956; Fennestad & Borg-Petersen, 1957; Penhale, 1965) that the calf is immunologically competent at birth and it would appear that it has the capacity to produce immunoglobulins of both IgG and IgM classes at this time. Moreover, relative to adult levels, IgM synthesis would seem to be more efficient at this stage than IgG.

In conclusion, the present experiments clearly show that colostrum-deprived calves can be protected against experimental challenge with a highly virulent invasive strain of E.coli by the administration of relatively small amounts of

immunoglobulin during the early neonatal period and also provide further evidence that the immunoglobulins of the IgM class are the most significant in this respect. In this connection, it is of interest to note that those calves which survived as a consequence of the administration of the serum IgM fraction had plasma IgG levels approximately similar to those of market calves which invariably died of septicaemia (Penhale & Christie, McEwan, Fisher & Selman, 1970).

CHAPTER VIII

In preceding chapters, it has been demonstrated that either colostral whey or IgM rich fractions, prepared from serum, could prevent septicaemia when administered parenterally prior to experimental infection of colostrum deprived calves. However, in these calves there was a high incidence of enteric disease as manifested clinically by severe diarrhoea which appeared to be little influenced by the prophylactic measures used. In view of this, and since colostrum, when acquired naturally by ingestion, can afford protection against both forms of colibacillosis, i.e., septicaemia and enterotoxic diarrhoea, it was suggested that the immunity was of a complex nature involving two separate systems; (1) systemic - mediated largely by IgM preventing septicaemia and (2) local - within the lumen of the small intestine, inhibiting enteric disease. Such a local passive immunity has been shown to occur in transmissible gastro-enteritis in piglets (Hooper & Haelterman, 1966). Moreover, in other species, local immunity of an active nature has been recorded. For example, Davies (1922) observed the presence of specific antibodies in the faeces of patients suffering from dysentery and during the late 1940's, in a series of experiments, Burrows and his co-workers (Burrows, Elliott & Havens, 1947; Burrows & Havens, 1948; Burrows, Deupree & Moore, 1950 a & b) demonstrated that there was a correlation between local intestinal antibody and protection against experimental infection in guinea pigs challenged with Vibrio cholerae. Furthermore, they observed that animals which

had high serum antibody titres were resistant to intracerebral challenge but were no more resistant to oral infection than controls. Thus it became apparent that serum and local immunity were independent and this had been confirmed by other workers in different diseases (Kerr & Robertson, 1953; Kerr, 1955; Byrne & Nelson, 1939).

To date such a local immunity has not been demonstrated in colibacillosis in calves.

With the ability to prevent colisepticaemia without affecting to any great extent the diarrhoeic syndrome it was now possible to investigate the intestinal role of colostrum in relation to purely enteric disease. This was done by providing calves initially with systemic protection against septicaemia, followed by the oral administration of whey which was delayed until absorption could no longer occur from the small intestine. This ensured that the colostral immunoglobulins remained within the gastro-intestinal tract. The effect of this procedure was assessed by comparison with calves receiving only systemic immunoglobulin.

MATERIALS & METHODS.

It was not possible to obtain colostrum deprived calves for this experiment so market calves with only minimal serum immunoglobulin levels were used.

The calves, between 3 and 7 days old, were divided into 4 groups, as follows:-

1. Control calves which received no prophylactic treatment.

2. Calves which were given an IgM fraction intravenously (1·0g. IgM/30 kg. bodyweight).
3. Calves which were given similar quantities of the IgM fraction and 3 hours later 500 ml. of pooled colostral whey orally and thereafter daily doses of 150, 200, 200 and 120 ml. of whey (Fig. 36).
4. Calves which were given only whey orally in doses similar to group 3.

The calves were then placed collectively in premises which had been contaminated by the calves used in earlier studies. They were not experimentally infected, colibacillosis being allowed to develop spontaneously.

Calves.

The calves were purchased from a dealer who had bought them at two markets in the West of Scotland. Using a zinc sulphate turbidity test to assess their serum immunoglobulin levels, they were screened and those with minimal readings, i.e., less than 7 units, were used in the experiment. It was known from the results of Gay, Anderson, Fisher and McEwan (1965) that calves with readings of less than 10 units were markedly or absolutely deficient in immunoglobulin and were susceptible to septicaemia.

On arrival, they were weighed, blood samples were taken for bacteriological and serological examination, and thereafter, twice daily throughout the experiment. Similarly, temperature, pulse and respiratory rates were measured. A daily faeces

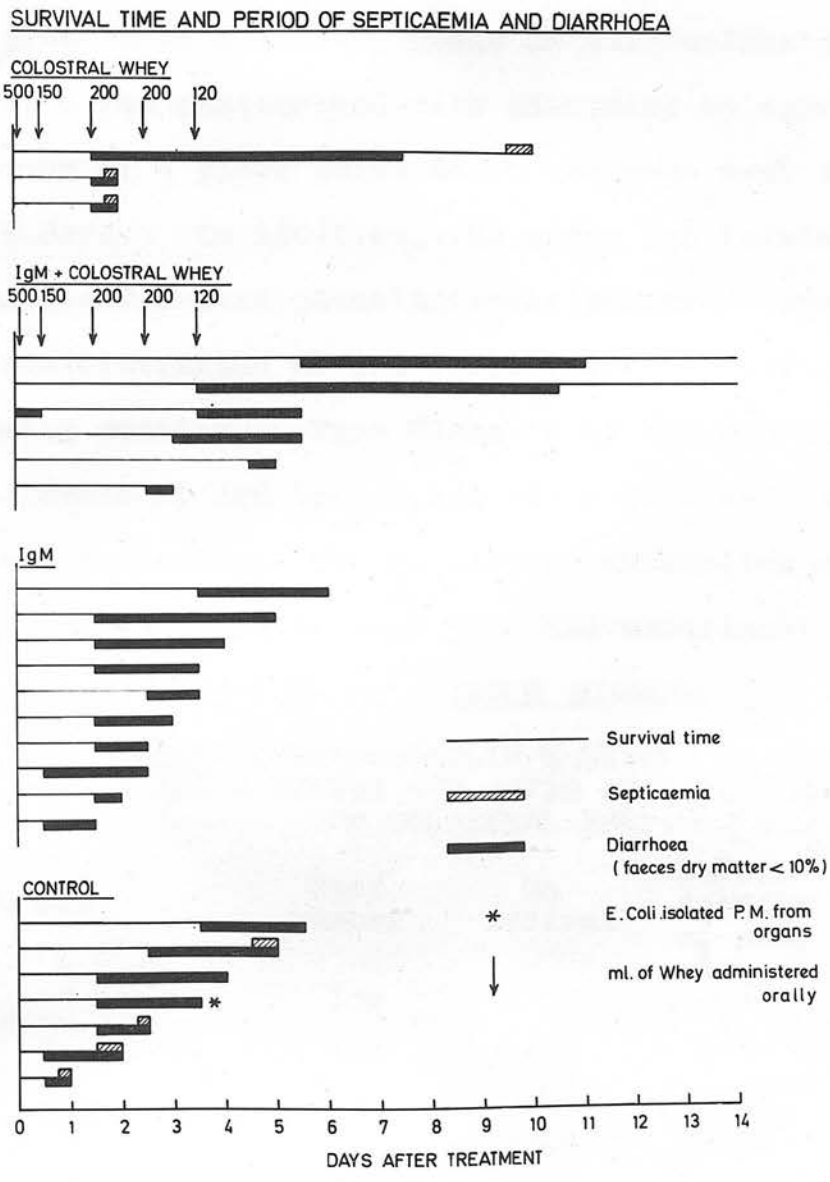

FIGURE 36

Survival time and periods of diarrhoea and septicaemia in the different groups of calves.

sample was taken from the rectum and dried to constant weight.

The calves were then divided into their respective groups, each group with a similar range of zinc sulphate readings. They were fed pasteurised milk according to appetite, up to a maximum of 4 pints twice daily and were kept under observation for 14 days. In addition, the serum IgG levels of calves in groups 3 and 4 were quantitatively measured immediately before oral administration of the first doses of whey and again the following morning. From Table XI it can be seen that the serum levels of IgG before and after whey had been given were similar, indicating that no further absorption of immunoglobulin, which would have interfered with the experiment, had occurred.

TABLE XI

SERUM IMMUNOGLOBULIN G LEVELS IN GROUPS 3 AND 4 BEFORE AND AFTER INITIAL FEEDING OF COLOSTRAL WHEY.

Group	Calf number	On arrival	Before feeding of whey	12 hrs. post feeding of whey
3 (IgM + Colostral whey)	162	0·19	0·41	0·30
	166	2·40	2·40	2·40
	167	0·24	0·51	0·41
	175	0·50	0·86	0·70
	180	0·80	0·64	0·71
	174	2·10	1·45	2·00
4 (Colostral whey)	163	0·26	0·33	0·20
	165	0·19	0·18	0·10
	181	0·17	0·21	0·20

Preparation of Colostral Whey.

Colostrum taken at the first post-partum milking was collected from various farms. After pooling, fat was removed by centrifugation and whey was prepared as described in Chapter II.

Zinc Sulphate Turbidity Test.

This was carried out using the method of McEwan, Fisher, Selman and Penhale, (1970) but the turbidity readings were made with an EEL (Evans Electroselenium Ltd.) spectrophotometer. (McBeath, Penhale & Logan, 1971).

Bacteriology.

Twice-daily, blood samples and swabs taken from various tissues of calves which died were examined bacteriologically for the presence of organisms. Isolated E.coli were tested by slide agglutination against standard antisera to serotype O78K80(B).

RESULTS.

2 calves were found on arrival to be septicaemic and were excluded from the experiment. The observations on the remaining calves are summarised in Fig.36.

Of the 26 calves used in the experiment, only 2 which were given both IgM and colostral whey survived the observation period. Diarrhoea developed quickly, particularly in the control calves and also those given only IgM fraction, the first calf dying within 24 hours of being placed in the infected premises. Of the calves which were given IgM fraction

intravenously, none became septicaemic, whereas of the other 10 calves, 8 became septicaemic. E.coli serotype O78K80(B) which had been used extensively in these premises in earlier experiments was isolated from only 1 of the septicaemic calves.

1. Control Calves.

All 7 calves in this group died, the mean survival time being 3·3 days (1 to 5·5 days). The calves began to scour shortly after being placed in the premises (Fig.36) and once diarrhoea was established there was a rapid deterioration in condition. Terminally there was marked dehydration with haemoconcentration, the P.C.V. increasing by between 20 and 50 per cent (Fig.37). Most calves showed intermittent pyrexia, there being a rise in temperature of 2 - 3°F.

E.coli were isolated from the peripheral blood of 4 calves, and on post mortem examination, from these calves and a further calf, E.coli were cultured from the tissues. In only one case was the E.coli isolate identified as being serotype O78K80(B) (Appendix VI).

2. Calves given IgM Fraction Intravenously.

10 calves were each given IgM intravenously. All calves died; the mean survival time being 3·4 days (1·5 to 6 days). Clinically, these calves were similar to the control group, but E.coli were neither isolated ante-mortem from the peripheral blood nor at post mortem examination, from the organs. However, large numbers of mucoid E.coli were regularly found in the upper small intestine and in some mesenteric lymph nodes (Appendix VI). Again there was marked haemoconcentration (Fig.37).

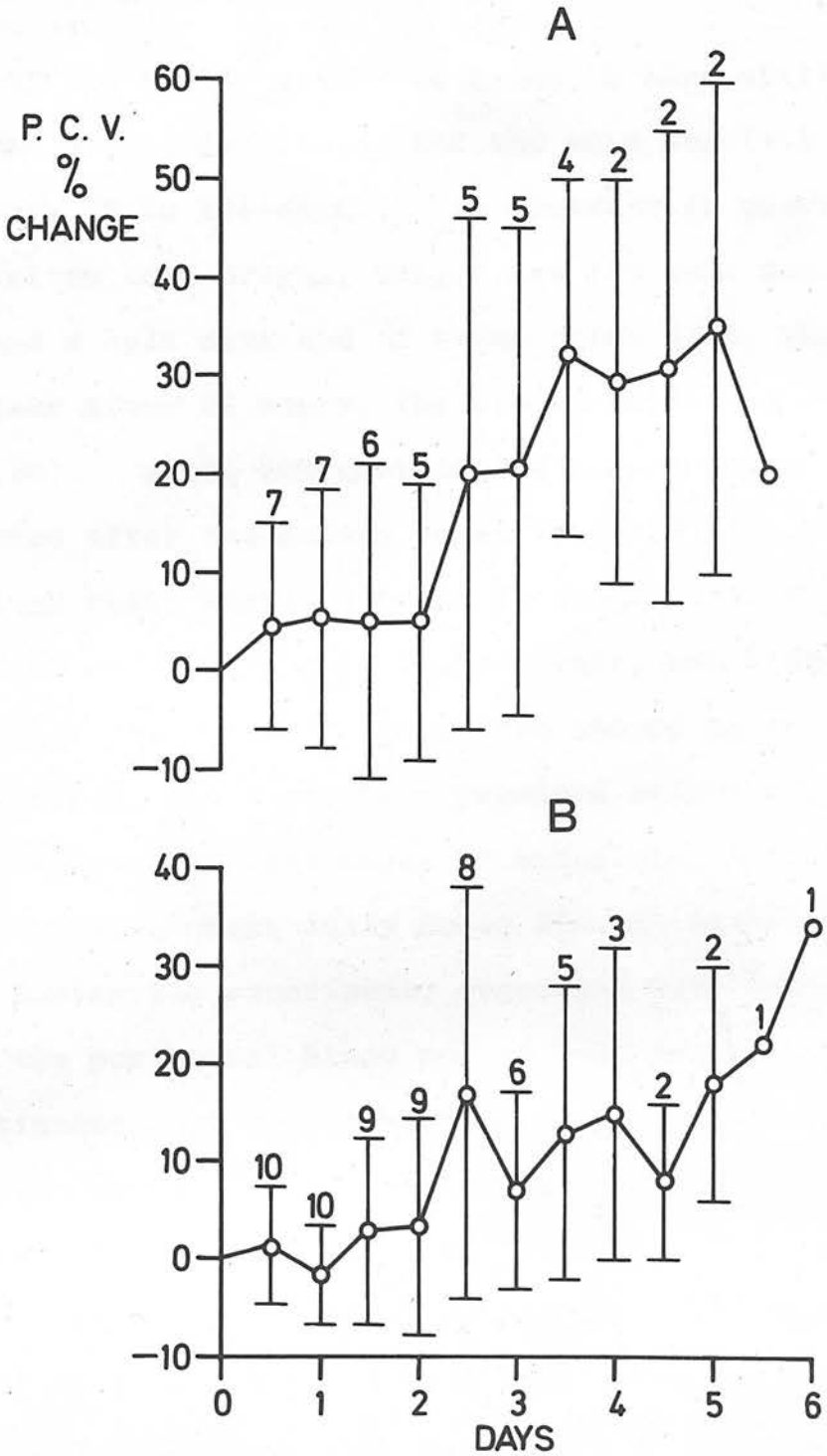

FIGURE 37

Daily changes with standard deviation (S.D.) in packed cell volume of A control calves B IgM treated calves. Above S.D. are numbers sampled.

3. Calves given IgM Fraction Intravenously and Colostral Whey Orally.

Of the 6 calves in this group, 2 were still surviving at the end of the experiment and the mean survival time was 7·8 days (3 to 14+ days). In contrast to previous groups, the calves were bright, hungry and fed well for the first two and a half days and of those which died, the first began to scour after 60 hours, the others following at intervals (Fig.36). Again dehydration and haemoconcentration were observed after the calves began to scour (Fig.38). The 2 calves which survived began to scour after three and a half and five and a half days respectively, and although there was prolonged diarrhoea, these calves showed no evidence of dehydration, and throughout remained bright and hungry. 2 of those which died began to scour whilst still receiving whey and subsequent daily doses did not alleviate the diarrhoea.

During the experiment, organisms were never isolated from the peripheral blood nor at post mortem examination from the tissues, but mucoid strains of E.coli were again cultured from the proximal small intestine (Appendix VI). Several of these mucoid strains of E.coli isolated from the small intestine and mesenteric lymph nodes of the non-septicaemic diarrhoeic calves in groups 2 and 3 have been shown to belong to the known enterotoxigenic strains "B42", "B85", "B117" and B111" as originally identified by Smith and Halls (1967a).

4. Calves given Whey Orally.

3 calves were fed whey orally in doses similar to that of

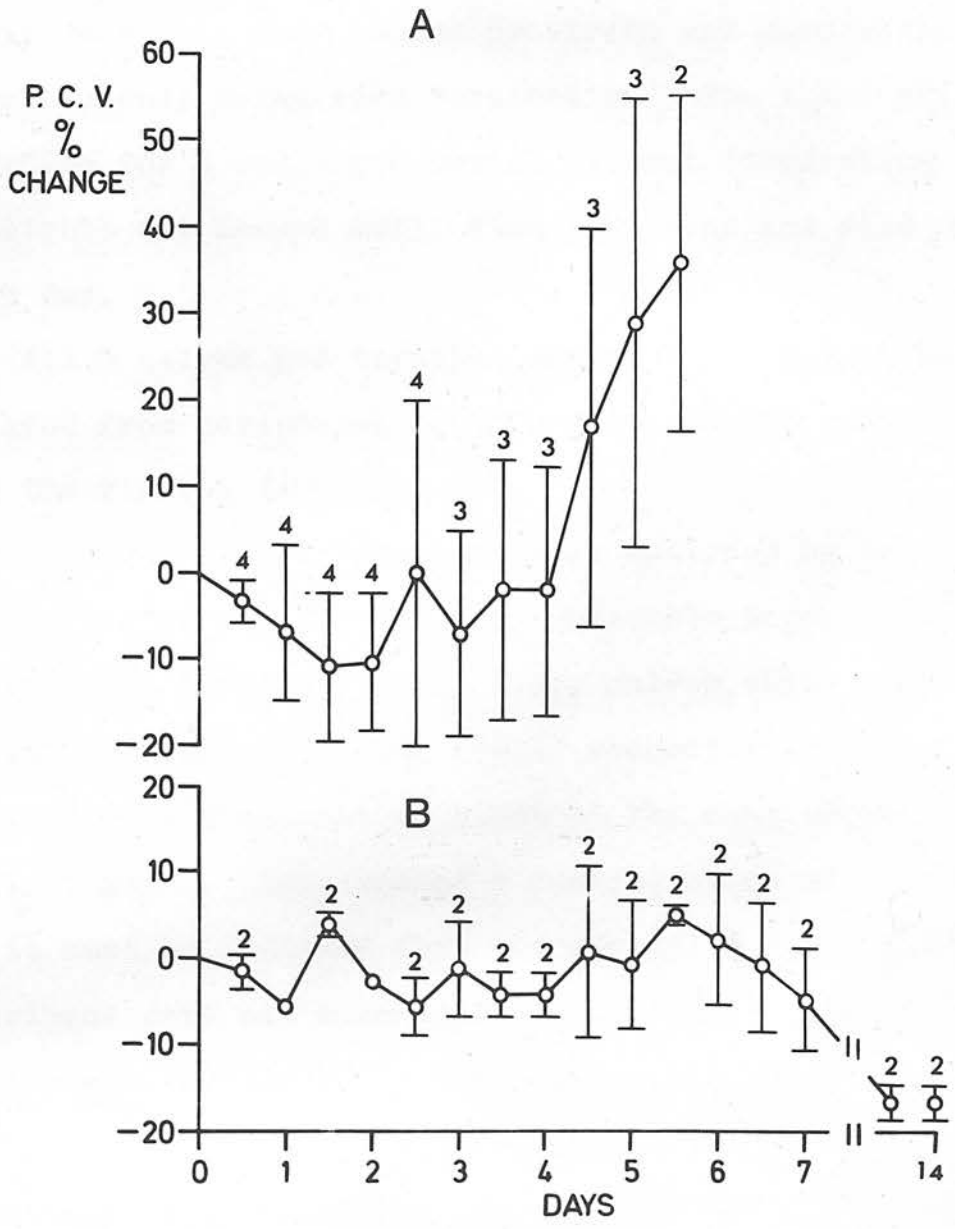

FIGURE 38

Daily changes with standard deviation (S.D.) in packed cell volume of the group of calves receiving both IgM fraction and colostral whey A dead calves B survivors. Above S.D. are numbers sampled.

group 3 but without the IgM fraction parenterally. Within 24 hours, 2 of the calves showed pain and stiffness of the stifle joints. They became prostrate and died within 2 days, diarrhoea only being seen terminally. The third calf had diarrhoea for a prolonged period without dehydration but on the eighth day became dull, then recumbent and died on the tenth day.

All 3 calves had terminal septicaemia, E.coli being isolated from peripheral blood and at post mortem examination from the tissues (Appendix VI).

The results were statistically analysed by randomisation test. The survival times, and intervals before the onset of diarrhoea in groups 2 and 4, i.e., calves which received only IgM intravenously and whey orally respectively, were not significantly different to those of the control calves, group 1. At first glance, this seemed a contradiction of earlier results, but it must be realised that the control calves in this experiment were not agammaglobulinaemic and thus in these septicaemia was partially inhibited. In the calves of group 3 which received both IgM fraction and whey, the survival time was significantly prolonged ($p < 0{\cdot}02$) and the onset of diarrhoea was delayed ($p < 0{\cdot}007$). The administration of IgM fraction to groups 2 and 3 reduced the incidence of septicaemia ($p < 0{\cdot}005$); of the 16 calves in these groups none developed septicaemia. In contrast, of the 10 calves which were not given the IgM fraction, 8 subsequently developed septicaemia.

DISCUSSION.

As 5/7 of the control calves died of colisepticaemia and the remaining 2 with diarrhoea, it can be assumed that all the calves were exposed to, and susceptible to, colibacillosis and that any significant difference in the inter group results could reasonably be attributed to the prophylactic treatment given.

In view of the clinical signs of scour, dehydration and haemoconcentration, which accompanied septicaemia in the majority of calves in the control group, it is likely that these calves, apart from the one which died within 24 hours, suffered from a septicaemic/enteric disease complex. It was interesting to note that although 3 weeks previously the premises had housed calves which had been experimentally infected with E.coli serotype O78K80(B), this serotype was found on only one occasion and this would suggest that the calves carried with them the other septicaemic strains isolated. This was supported by the finding that 2 calves were septicaemic on arrival.

The IgM fraction inhibited septicaemia but again failed to influence to any marked extent the enteric syndrome and it would appear that systemic antibodies play little part in this localised condition. That only one dose of IgM was necessary in market calves to prevent septicaemia was probably due to the fact that these calves were not newborn and already had

had minimal amounts of circulating antibody.

As before, it was believed that the diarrhoea seen in these calves was associated with the presence in the small intestine of large numbers of mucoid E.coli which were demonstrated as being enterotoxigenic strains.

In the group given both IgM and colostral whey orally, again septicaemia was prevented and in addition, the onset of scour was significantly delayed. The assumption that colostral whey would not be absorbed because of the age of the calves was subsequently confirmed by examination of sera for immunoglobulin. It is therefore concluded that the colostral whey immunoglobulin must have acted locally within the gastro-intestinal tract to inhibit enteric disease. This is in accord with other studies demonstrating that immunoglobulins can provide a local protective action within the gastro-intestinal tract (Davies, 1922; Burrows, et al 1947; 1948; 1950a; 1950b; Hooper & Haelterman, 1966; Kohler, 1967). Recent studies in other species (Tomasi, Tan, Solomon & Prendergast, 1965; South, Cooper, Wollheim, Hong & Good, 1966; Berger, Ainbender, Hodes, Zepp & Hevizy, 1967; Porter, Noakes & Allen, 1970b) indicate that IgA is the principal immunoglobulin providing protection at the paramucosal surface and it is probable that in the calf colostral IgA has a similar function. However, in the neonatal calf, protection is passive and other colostral immunoglobulins, in addition to IgA may contribute to this immunity.

The death from colisepticaemia of the calves which were only given whey orally further supports the conclusion that the colostral immunoglobulin administered was not absorbed from the intestine. Because of the short survival time of 2 of these calves, it was not possible to assess the influence of the whey on diarrhoea in this group.

In conclusion, these results indicate that two separate immunological systems are required to protect the neonatal calf against colibacillosis, one providing systemic protection the other local, and for survival in a contaminated environment both must be present in adequate proportions.

CHAPTER IX.

Throughout these studies a large proportion of both colostrum-deprived and market calves have died with an acute non-septicaemic diarrhoea. From the proximal small intestine and mesenteric lymph nodes of all these calves, mucoid strains of E.coli have been isolated in almost pure culture and it has been postulated that these organisms were responsible for the severe diarrhoea seen in these calves. This hypothesis is supported by the evidence that several of these strains of E.coli have been classified as belonging to known entero-toxigenic serotypes. E.coli are presently classified as being enterotoxigenic and thus potentially enteropathogenic if they cause dilation when injected into ligated loops of rabbit intestine (Smith & Halls, 1967a). Since the diarrhoeic syndrome so frequently occurred spontaneously, it might be possible to reproduce this condition experimentally using isolates from scouring calves. In the past, various workers have attempted to do so but with little success. Smith & Little (1927), McEwen (1950), Smith (1962) and Gay, McKay & Barnum (1964c) all failed to reproduce the condition. If colostrum-deprived calves were used, these generally died of septicaemia (Gay, McKay & Barnum, 1964c) whilst colostrum fed calves were refractory (Smith, 1962). Subsequently, Smith & Halls (1967a) showed that calves under 20 hours old, when given certain strains of E.coli developed diarrhoea of varying degrees of severity but it is not certain from these experiments that the calves were suffering from fatal non-septicaemic enteric disease,

as in most cases, the calves were killed within a short time of challenge.

By the use of IgM parenterally in the calf, it is possible to produce a unique situation where the occurrence of coli-septicaemia can be prevented whilst leaving the gastro-intestinal tract virtually unprotected. This therefore should provide a model for an attempt to reproduce experimentally the acute enteric syndrome which occurred spontaneously.

MATERIALS & METHODS

Experimental Procedure.

Colostrum-deprived calves were given IgM intravenously and subsequently challenged orally with enterotoxigenic E.coli. Total urine and faeces were collected daily and changes in blood chemistry were monitored. At post mortem examination, samples were taken for bacteriological investigation from a number of sites including the intestine and several mesenteric lymph nodes. Faeces and serum samples were investigated quantitatively for the presence of immunoglobulin of various classes.

Calves.

Ayrshire bull calves were collected from a closed herd within 24 hours of birth. On arrival, each calf was weighed and a serum sample taken and checked by immunoelectrophoresis to ensure that it was agammaglobulinaemic. Calves were injected intravenously with the IgM fraction. 1 gm. of IgM/30 kg. body weight was given initially and this was repeated on the

fourth day if the calf still survived. The calf was then placed in a restraining crate (Appendix VII) in order to obtain total faeces and urine output. Total faeces were collected by means of a latex rubber cone (Appendix VIII) moulded to fit tightly over the gluteal region and to the open apex of the cone was attached a nylon sleeve. Urine was collected in a receptacle beneath the crate. The experimental strain of E.coli was administered orally to each calf within 24 - 48 hours of birth.

Daily blood samples were obtained aseptically from the jugular vein. Because milk may contain low levels of immunoglobulins, (Mach & Pahud, 1971) the calves were fed twice daily with milk substitute (Lobol Gold Top Baby Calf Food) deficient in these components. This was reconstituted and fed according to the manufacturer's instructions (B.O.C.M. Silcock).

Escherichia coli.

E.coli serotype O101K(A?) was used throughout the experiment. The primary isolation of this strain was obtained from the small intestine of a market calf which had died with an acute diarrhoea. Prior to the commencement of the experiment the organism was lyophilised in a large number of 1 ml. ampoules and one of these was used as the inoculum for each calf.

When a calf became available the freeze dried organisms were subcultured in glucose broth for 6 hours. Each calf was given 10 mls. of undiluted culture orally.

Bacteriological Studies.

Blood samples and swabs taken from various organs were cultured on blood agar and MacConkey plates for 24 hours. A number of mucoid E.coli colonies cultured from the intestinal samples and mesenteric lymph nodes were tested by slide agglutination against antiserum specific for the challenge strain. Specific antiserum to O101K(A?) was prepared in a similar manner to the antiserum in Chapter II.

Faeces Examination.

Daily faeces samples were homogenised and divided into 2 aliquots, one being dried to constant weight, whilst the other was stored at -20°C for subsequent serological examination. 10 gm. of a homogenised sample was centrifuged to remove debris. If necessary a known quantity of PBS was added to dilute the samples. The supernatant was examined quantitatively for immunoglobulin of A, M and G classes by the technique of single radial immunodiffusion.

Preparation of IgA Antiserum.

IgA for the preparation of specific antiserum was prepared as follows. Colostral whey was used as the source of IgA; gammaglobulin was first precipitated using 28% (w/v) sodium sulphate solution and then fractionated by batch chromatography on DEAE - cellulose. IgG was first eluted using 0·02M phosphate buffer pH 6·5 and this was followed by 0·1M phosphate buffer pH 6·5 to elute the IgA containing fraction. This material was then further purified by recycling on Sephadex G 200 until

a single symmetrical peak was obtained. Antiserum to IgA was prepared in sheep, initially by giving intramuscular injections of the immunoglobulin in complete Freund's adjuvant followed by intravenous injections of the immunoglobulin alone. The resulting antiserum was rendered specific to IgA (Fig.39) by absorption with IgG, pre-colostral serum and free secretory piece prepared by the method of Mach (1971).

Blood Examination.

Plasma sodium and potassium were determined by flame photometry and chloride concentration by the method of Schales & Schales (1941). Blood urea nitrogen was measured by "Urastrat" chromatography paper. Packed cell volume was determined by the microhaematrocrit technique. Pre-challenged blood pH was regularly measured with a micro-electrode unit (Radiometer, Copenhagen) and whenever possible the pH of dying calves was also obtained within 3 hours of death.

RESULTS.

13 calves were challenged during the experiment and all suffered from severe diarrhoea. The first 5, of which 2 died, were challenged between 39 - 48 hours old, whilst the remaining 8 calves all of which died, were under 24 hours old. Culture of the routine blood samples showed that none of the calves was bacteraemic at any stage and this was confirmed by bacteriological examination of various organs post mortem.

Because of the marked differences in blood chemistry and certain clinical observations the calves can be conveniently

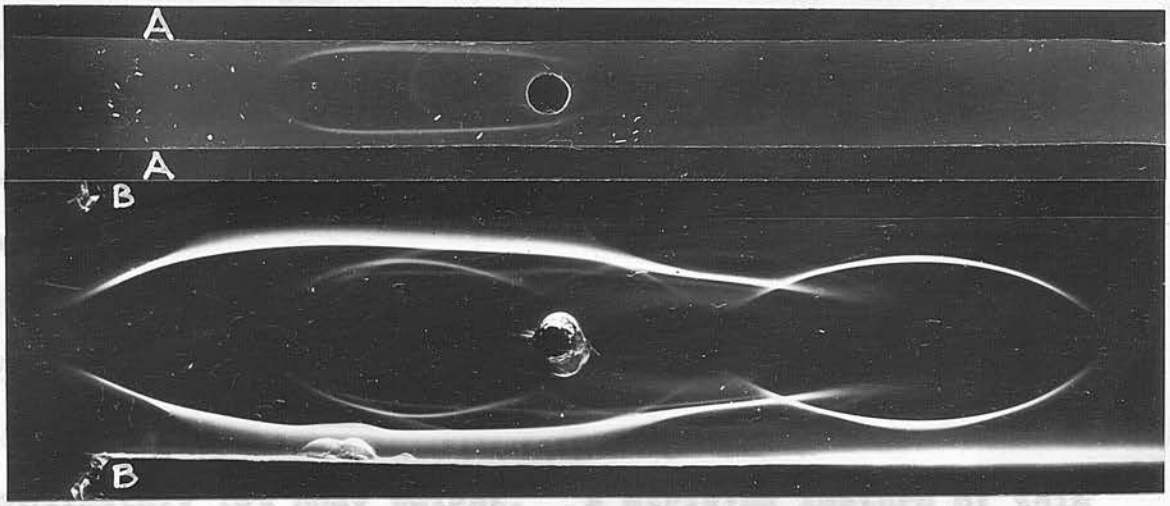

FIGURE 39

Immunoelectrophoresis of colostral whey using sheep anti-IgA serum.

Upper troughs A - sheep anti-IgA serum (absorbed)
Lower troughs B - rabbit anti-bovine serum
Wells - colostral whey.

grouped into those which died and those which survived.

Dying Calves.

All these calves developed severe diarrhoea within 24 hours of challenge, the faeces dry matter dropping rapidly to approximately 5% and at the height of the disease they were consistently voiding approximately 2 kg. of faeces daily (Fig. 40). Death occurred between the second and fourth day (mean 3·2 days) after challenge. Clinically, the calves quickly became dehydrated and developed sunken eyes (Fig. 41). Unlike the septicaemic calves in earlier studies, there was no early loss of muscle tone and the calves were able to stand until a short time before death. At death, each calf had lost approximately 16% body weight. A striking feature of this group was the marked oliguria, and later, terminal anuria. The effect of this syndrome on blood chemistry is summarised in Table XII. As the table shows, there was well marked ureamia and hyperkalaemia but with considerable individual variations depending on the survival time of the calf. Blood sodium and chloride ion concentrations were not altered and remained within the prechallenge range whilst blood pH was only slightly lowered. It was considered that the very marked haemoconcentration, where the P.C.V. was raised by as much as 50% above prechallenge level in some cases (Fig. 40) was highly significant as this trend was one of the earliest changes observed in the blood picture and was not merely a terminal finding.

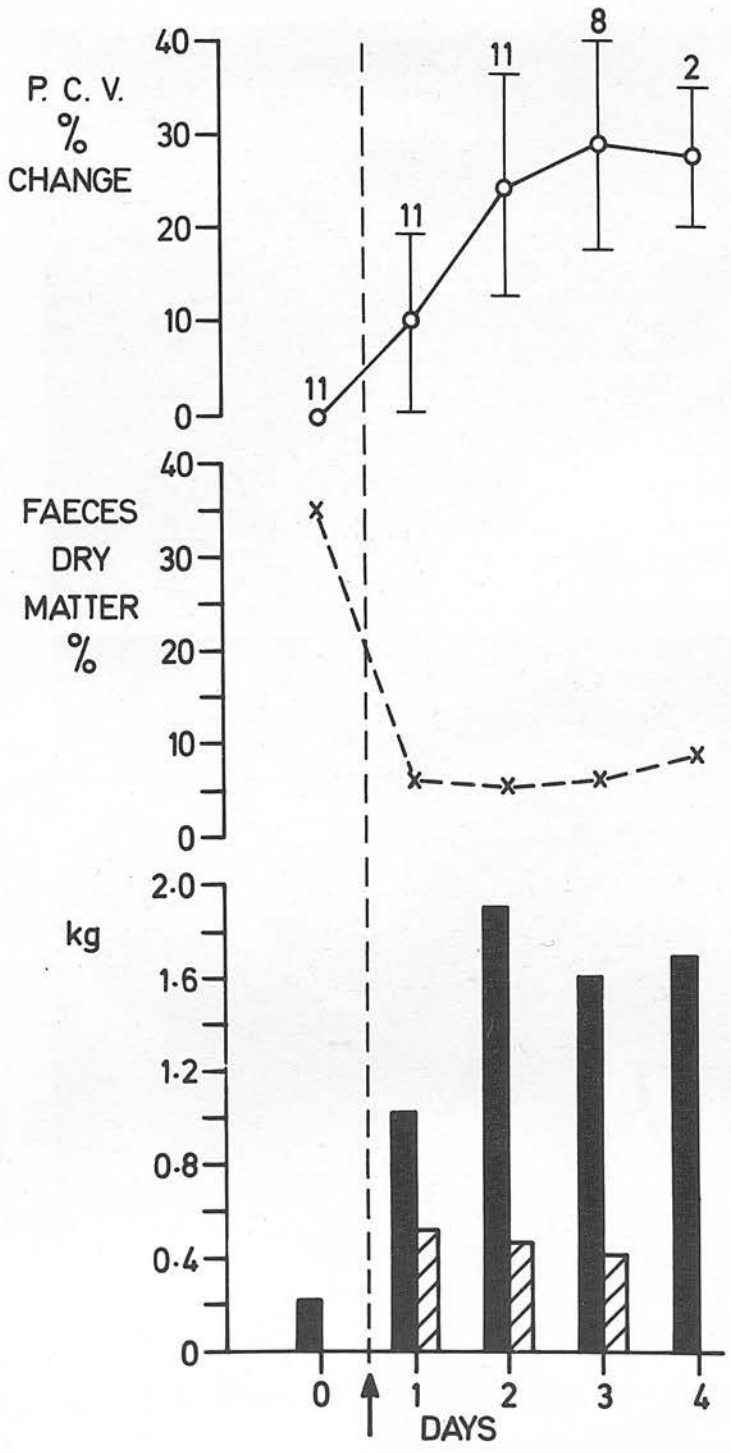

FIGURE 40

Dying calves; daily changes in packed cell volume (with standard deviation) and faeces dry matter. Daily faecal output, black column; daily urine output, hatched column. Above S.D. are numbers sampled. Arrow indicates time of challenge.

FIGURE 41

Top Normal healthy Calf.

Lower Dehydrated Calf.

Surviving Calves.

The 2 calves which survived also had severe diarrhoea within 24 hours of challenge (Fig. 42), but on the third day they began to recover and the daily weight of faeces voided dropped rapidly as the dry matter content increased. Unlike dying calves, urine output was not markedly diminished and there was no obvious dehydration. The P.C.V. and blood urea showed only a slight temporary rise but during the acute phase of the disease these calves had severe hyperkalaemia similar to that of the dying calves (Table XII).

At post mortem examination, as previously stated, in accordance with the negative findings obtained by blood culture, none of the calves was found to have E.coli in any of the tissues sampled. However, the challenge strain of E.coli was always isolated in large numbers throughout the small intestine and also from all the mesenteric lymph nodes examined (Appendix IX). This particular serotype of E.coli was also isolated from the diarrhoeic faeces of the surviving calves.

Immunoglobulin Levels in Faeces and Serum.

The immunological findings are summarised in (Fig. 43) IgG and IgM appeared in the faeces within 24 hours of challenge; IgA was isolated between 24 and 48 hours and all reached a peak when diarrhoea was most severe.

IgG and IgM were found to be present in serum as was to be expected following the dosage with immunoglobulin, but IgA was

TABLE XII

Changes in blood chemistry of Diarrhoeic Calves.

			Na^+ m.eq./litre	K^+ m.eq./litre	Cl^- m.eq./litre	Urea Nitrogen mg./100 ml.	pH	Wt. (kg.)
Dying Calves	Pre-challenge	Mean	140	4·8	108	14	7·42	38·3
		Range	132-150	4·3-5·7	97-114	10-17	7·36-7·53	
	at death	Mean	133	7·4	106	61	7·29 (6 calves)	32·7
		Range	127-147	4·8-9·8	90-129	37-94	7·21-7·36	
Surviving Calf	Pre-challenge		140	5·2	106	16		
	Range during illness		128-144	5·2-7·2	95-106	16-36		
Surviving Calf	Pre-challenge		136	5	103	12		
	Range during illness		120-136	4·0-6·75	100-108	12-25		

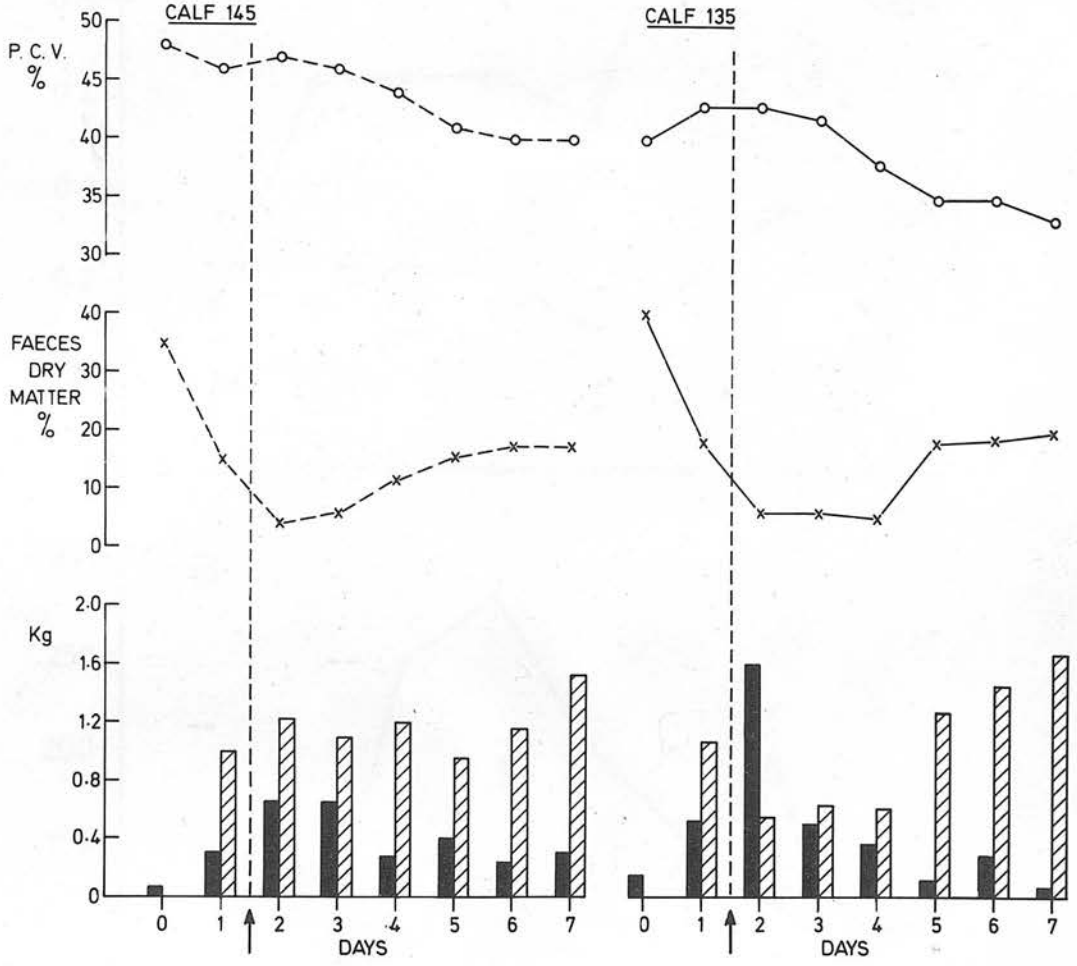

FIGURE 42

Surviving calves. Daily changes in packed cell volume, faecal dry matter and faeces and urine output in each calf. Daily faeces output, black column; daily urine output, hatched column. Arrow indicates time of challenge.

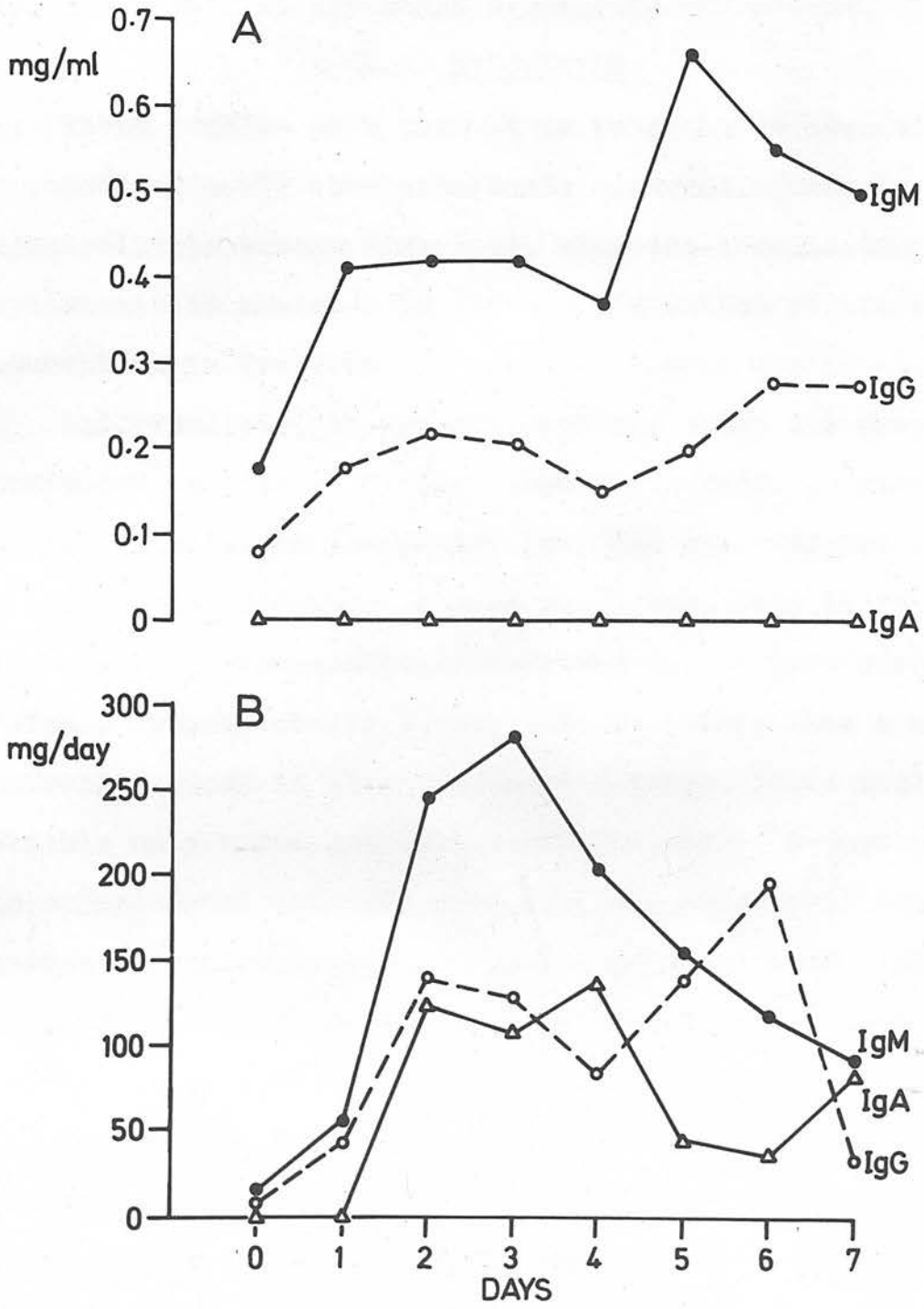

FIGURE 43

A Daily serum immunoglobulin levels.

B Daily immunoglobulin excretion in faeces.

not detected either by radial immunodiffusion or immuno-electrophoresis at any stage during the experiment.

DISCUSSION.

These results show that it is possible to consistently reproduce an acute non-bacteraemic syndrome, characterised principally by severe diarrhoea, when the complication of septicaemia is excluded by the administration of the requisite immunoglobulin fraction.

Unfortunately, it was not possible under the prevailing conditions to design an experiment which would provide normal control animals for comparison with the experimental group, as spontaneous diarrhoea always developed, even in the absence of challenge, when colostrum deprived calves were given only systemic immunoglobulin cover. It is likely that a completely controlled study of this aspect of colibacillosis would be possible only under gnotobiotic conditions. Nevertheless, the experimental serotype of E.coli was invariably the predominant organism isolated from the proximal small intestine of these calves and it is therefore considered that this syndrome is an acute form of enteric colibacillosis.

The present results are in agreement with the observations of Smith and Halls (1967a) that calves experimentally infected with E.coli when under 24 hours old are more susceptible than older calves. Since immunoglobulins appeared in the faeces as early as 24 hours after challenge it is possible that this resistance of older calves could be attributed to their presence.

It was not possible to decide whether these immunoglobulins are produced locally in the small intestine or are derived from the circulation. However, it would seem likely that this was the case with IgA as it was not possible to detect this particular class of immunoglobulin in any quantity in the plasma during this period by the techniques employed.

On examination of the various parameters, it was noted that the dying calves did not have the severe acidosis that has been described in older diarrhoeic calves (Fisher, 1965; Watt, 1967; Fayet, 1968 a & b) nor had they significantly higher plasma potassium levels than the present surviving calves (Fisher, 1965).

In the present study, it was considered that the most significant difference between surviving and dying calves was the marked increase in P.C.V. seen in the dying calves. Although in this particular experiment there were only 2 survivors, this difference has also been observed in the preceding studies using both colostrum deprived and hypogammaglobulinaemic calves. This marked increase may be due to the presence in the small intestine of some ultimately absorbable haemo-concentration factor, possibly endotoxin as previously suggested in Chapter II. It is possible that during the course of scouring this factor is absorbed into the circulation where it causes the haemodynamic changes leading to circulatory and renal failure observed in this group. Under normal circumstances intestinal immunoglobulins may have the ability either

to neutralise or block this factor.

CHAPTER X.

GENERAL DISCUSSION & CONCLUSIONS.

From the results of the present studies, it is clear that the immunoglobulin status of the calf is of prime importance in the pathogenesis of colibacillosis. By experimentally adjusting the immunity of the colostrum-deprived calf, it is possible to show that colibacillosis exists as two distinct syndromes, namely septicaemia and enteric disease. These can be reproduced as separate entities although in the field they frequently occur together as a septicaemia/scour complex. Whilst both syndromes are caused by Escherichia coli, different strains are involved in each case. Serum immunoglobulin and in particular serum IgM is necessary for the protection of the calf against septicaemia but apparently has little significance in enteric disease. Serum IgM acts by preventing invasive strains of E.coli entering the circulation with the resultant release of endotoxin. In the present experiments once the organism which was particularly virulent established itself in the blood, the calves never survived in spite of the fact that there was a specific serum IgM response. Indeed, in certain cases, the immune response appeared to accelerate death (vide infra). Smith & Halls (1968a) demonstrated that even colostrum-fed calves were unable to withstand the effects of colisepticaemia if injected intravenously with E.coli organisms of the serotype 078K80(B).

Two interesting findings in relation to septicaemia were that (1) .25g of IgM as present in whey was sufficient to

protect calves as compared with 2g. of IgM as euglobulin (2) bacteraemia could occur terminally either over two or three days or as peracute form during the last 12 hours of life. Neither of these findings have been investigated. In the former, it may be that the IgM in colostrum had either greater biological activity or different specificity or had a longer half life than that in the euglobulin preparation where some denaturation could occur during fractionation. A longer half life or increased specificity would permit lower doses to maintain adequate serum antibody levels.

Initially, it was postulated that the different types of bacteraemia observed in the calves which died of septicaemia might have been associated with slowness of absorption of immunoglobulin from the peritoneum. However, as the two types also occurred in calves receiving IgM intravenously, this hypothesis can probably be discounted. It is possible that the nature of the bacteraemia is related either to the time of re-infection of the calf from its environment or to variations in the immune response and competence of individual calves. In the peracute form which was accompanied by severe depression and collapse it may be that the immune response was detrimental to the calf in that the destruction of massive numbers of organisms by the antibody complement mechanism would cause the release of large volumes of endotoxin to which calves are particularly susceptible.

It has been demonstrated that colostral whey has a local

protective action in the intestines and this has been attributed to the immunoglobulins present in the whey. The effect of administering whey orally to market calves was not as conclusive as one would have anticipated in that 2 out of 6 calves survived and all suffered diarrhoea to some degree. Since the calves were several days old it is likely that intestinal infection may have been present in some of these calves prior to administration of the whey. This could result in bacteria being able to adhere to the mucosa and produce enterotoxin before an immunological barrier had been established. The fact that whey, given to one scouring calf had no ameliorating effect on the diarrhoea, would support this hypothesis and studies in progress have confirmed that the protective value of colostrum is lost if feeding is delayed until after calves have been experimentally infected. Rutter & Anderson (1971) have demonstrated a similar finding in intestinal infection in piglets. It is therefore probable that colostrum has a prophylactic effect rather than a curative one and thus must be present in the small intestine prior to infection. Intestinal immunoglobulin would appear to have at least two effects:- either it prevents enteric disease completely or failing that it prevents in some as yet unknown manner the marked haemo-concentration which was a feature of dying diarrhoeic calves. It may be that these are simply quantitative differences in colostral function but on the other hand they may represent qualitative differences in individual classes of immunoglobulin.

When the calves were experimentally infected with enteropathogenic strains of E.coli, immunoglobulins G, A and M appeared in the faeces within 48 hours and reached a peak at the time when diarrhoea was most severe. It is possible but unlikely, that these immunoglobulins were derived from the serum because IgA could not be identified in the sera of calves during the period of the experiment yet was present in the faeces. Moreover, if faecal IgM had leaked from the circulation it could be expected that the mean loss of approximately 800 mg. of faecal IgM in four days out of a total of 1 gm. administered would have produced a marked drop in serum IgM levels. This did not occur and indeed serum levels of IgM were very similar to those of the double dose group of calves in which scour was minimal. At present, the role of individual immunoglobulins is being investigated and preliminary results suggest that the protective activity of colostrum is not confined to one immunoglobulin but is present in both IgA and IgM.

The concept of a local independent intestinal immunity is an apparent contradiction of earlier findings (Gay, Anderson, Fisher & McEwan, 1965; McEwan, Fisher & Selman, 1970) that mortality and the type of disease seen in any calf is directly related to serum immunoglobulin levels as measured by the zinc sulphate turbidity test. The present results only support this hypothesis as far as septicaemia is concerned and it is probable that in hypogammaglobulinaemic calves which rarely receive more than one feed of colostrum before being sold that serum

immunoglobulin levels are an indirect measurement of intestinal levels. In other calves, e.g., suckled calves, which receive colostrum over the first few days of life, intestinal levels of immunoglobulin would be very much higher and would bear little relationship to serum levels.

With the ability to reproduce enteric colibacillosis it will be possible to examine in greater depth the pathogenesis of this condition. In particular, it will be possible to investigate the dehydration and intense haemoconcentration which occurred in the dying calves. Fisher (1972) has suggested that haemoconcentration in scouring calves does not occur until the calves have stopped feeding and therefore are losing fluid which is not being replaced. In the experiments reported here, haemoconcentration was one of the first changes observed and always preceded loss of appetite. In his experiments, Fisher was using market calves which were several days old, whereas newborn colostrum deprived calves were used in these experiments and so the different results obtained may be a reflection of the immunoglobulin status of the two groups of calves.

Finally, from the studies reported in this thesis, it is concluded that some of the most significant findings are that 1) The immunity of the calf to colibacillosis is of a complex nature involving two separate independent systems, namely systemic, preventing septicaemia by certain strains of E.coli and local, within the small intestine, inhibiting the local enteropathogenicity of other E.coli strains.

2) Immunoglobulin of the IgM class is the principal immunoglobulin with activity against septicaemic strains of E.coli.

3) The presence of E.coli in the blood of calves stimulates a specific IgM response.

4) 2g. IgM, isolated from pooled bovine serum can prevent septicaemia when given intravenously in two doses at an interval of four days.

5) The half life of IgM is quite short, approximately 3 - 4 days.

6) Certain strains of E.coli can act as primary pathogens in the small intestine and cause diarrhoea and death.

7) Severe haemoconcentration accompanied by oliguria is an important factor in the pathogenesis of acute enteric colibacillosis.

8) Calves are immunologically competent at birth and can produce immunoglobulins of the IgM, IgG and IgA classes at this time.

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APPENDIX I

SUMMARY OF BACTERIOLOGICAL FINDINGS IN CALVES RECEIVING COLOSTRAL WHEY

Daily Examination of Peripheral Blood

CALF	0 PM	1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM	15 AM	15 PM	16 AM	16 PM	17 AM	17 PM	18 AM	18 PM	19 AM	19 PM	20 AM	20 PM	21 AM	21 PM
2	-	-	-	-	+	+																																					
3	-	-	-	-	-	-	-																																				
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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16	-	-	-	-	-	-	-	-	-																																		
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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22	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+																									
23	-	-	-	+	+	+	+																																				
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29	-	-	-	-	-																																						
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31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+																						
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35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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41	-	-	-	-	-	-	-	-																																			
42	-	-	-	-	-	-	-																																				
43	-	-	-	-	-	-	-	-																																			

\+ = *E. Coli* 078K80(B)

o = *E. Coli* NOT 078K80(B)

Post Mortem Examination

CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	STIFLE JOINT	SMALL INTESTINE
2	+	+	+	+	+	+	+	o
3	-	-	-	-	-	-	-	o
8								
9								
10								
11	-	-	-	-	-	-	-	o
12	-	-	-	-	-	-	-	o
14	+	+	+	+	+	+	+	o
15	+	+	+	+	+	+	+	o
16	+	+	+	+	+	+	+	o
17								
18								
19	o	o	o		o	o	-	o
20	-	-	-	-	-	-	-	o
21	+	+	+	+	+	+	+	o
22	+	+	+	+	+	+	+	o
23	-	+	+	-	+	+	-	o
24	-	-	+	-	+	-	-	o
25	+	+	+	+	+	+	-	o
26	-	-	-	-	-	-	-	o
27	-	-	-	-	-	-	-	o
28								
29	-	-	-	-	-	-	-	o
30	+	+	+	+	+	+	+	+o
31								
32								
33	+	+	+	+	+	+	+	o
34	o	o	o	o	o	o	o	o
35								
36	-	-	-	-	-	-	-	o
37	-	-	-	-	-	-	-	o
39	+	+	+	+	+	+	+	o
40	+	+	+	+	+	+	+	o
41	-	-	-	-	-	-	-	o
42	-	-	-	-	-	-	-	o
43	+	+	+	+	+	+		o

A P P E N D I X II

SUMMARY OF BACTERIOLOGICAL FINDINGS IN CALVES RECEIVING IgG IgM AND CONTROL CALVES

Daily Examination of Peripheral Blood																Post Mortem Examination								
CALF	0 PM	1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	STIFLE JOINT	SMALL INTESTINE
IgG														IgG								
65	-	-	+1	+1		+2	+3	+4						65	+	+	+	+	+	+	+	o
64	-	-	-	-	-	-	-	+1	+2	+3	+4			64	+	+	+	+	-	+	+	o
24/93	-	-	-	-	-	+2	+3	+3	+4					24/93	+	+	+	+	+	+	+	o
63	-	-		+3	+4									63	+	+	+	+	+	+	+	o
84	-	-		+2	+3									84	+	+	+	+	+	+	+	o
IgM														IgM								
69										+4				69	+	+	+	+	+	+	+	o
70										+2	+4			70	+	+	+	+	+	+	+	o
75										+4	+4			75	+	+	+	+	+	+	+	o
82								+1	+4					82	+	+	+	+	+	+	+	o
CONTROLS														CONTROLS								
1				+2	+4	+4	+4							1	+	+	+	+	+	+	+	o
4			+1	+2	+3	+4								4	+	+	+	+	+	+	+	o
13			+3	+4										13	+	+	+	+	+	+	+	+
31				+1	+1	+2	+4	+4						31	+	+	+	+	+	+	+	o

\+ = *E. Coli* 078K80(B)

o = *E. Coli* NOT 078K80(B)

1 = 1 - 10 Colonies *E. Coli* 078K80(B) /ml

2 = 10 - 100 Colonies *E. Coli* 078K80(B) "

3 = 100 - 1000 Colonies *E. Coli* 078K80(B) "

4 = > 10,000 Colonies *E. Coli* 078K80(B) "

Terminally, in order to count bacteria it was necessary to dilute blood samples 1:10 with sterile normal saline.

APPENDIX III

SUMMARY OF BACTERIOLOGICAL FINDINGS IN CALVES RECEIVING 1M FRACTION

Daily Examination of Peripheral Blood

CALF	0 PM	1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM
4	-	-	-	-	-	-	-	-	-	+1	+2	+3																	
44	-	-	-	-	-	-	-	+1	-	+3																			
45	-	-	-	-	-	-	-																						
46	-	-	-	-	-	-	-	+1	-	+1	+2																		
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	-	-	-	+2	+2	+3	+4															
51	-	-	-	-	-	-	-	-	-	-	-	+1	+1	+2	+3	+4													
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	-	-	-	-	-																								
54	-	-	-	-	-	-	-	+2	+2	+2	+2	+2	+2	+2	+3	+3	+4												
55	-	-	-	-	-	-	-	-	+4																				
56	-	-	-	+2	+3	+4																							
66	-	-	-	-	-	-	-	-																					
67	-	-	-	-	-	-	+2	+3	+4																				
68	-	-	-	-	-	-	-	-	-	-	-																		
71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-	-	-	-	+1	+1	+2	+2	+2	+3	+4											
74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-	-	-	-	-	+2	+4															
77	-	-	-	-	-	-	-	-	-	-	-	+2	+4																
79	-	-	-	-	-	-	-	-	-	-	-																		
81	-	-	-	-	-	-	-	-	+2	+3	+4																		
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
103	-	-	-	-	-	-	-	-	-	-	-	-	+1	+3	+4														
104	-	-	-	-	-	-	-	-	-	-	-	-																	
109	-	-	-	-	-	-	-	-	-	-	-	-	-	+1	+2	+2	+3	+3											
110	-	-	-	-	-	-	-	-	-	+1	+4																		
111	-	-	-	-	-	-	-	-	-	+1	+2	+3	+4																
CONTROLS																													
A	-	-	+2	+3	+4																								
B	-	+4																											
C	-	-	-	+1	+2	+3	+4																						
D	-	+1	+2	+2	+2	+3	+4																						
E	-	-	+1	+3	+4																								
F	-	+2	+4																										
G	-	+4																											

+ = *E. Coli* 078K80(B)

o = *E. Coli* NOT 078K80(B)

1 = 1 - 10 Colonies *E. Coli* 078K80(B)/ml

2 = 10 - 100 Colonies *E. Coli* 078K80(B) "

3 = 100 - 1000 Colonies *E. Coli* 078K80(B) "

4 = > 10,000 Colonies *E. Coli* 078K80(B) "

Post Mortem Examination

CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	STIFLE JOINT	SMALL INTESTINE	MESENTERIC LYMPH NODES 1	2	3	4	5	6
4	+	+	+	+	+	+	+	o						
44	+	+	+	+	+	+	+	o						
45	-	-	-	-	-	-	-	o	o	o	o	o	o	o
46	+	+	+	+	+	+	+	+						
47														
48														
49	+	+	+	+	+	+	+							
51	+	+	+	+	+	+	+							
52														
53	-	-	-	-	-	-	-	o	o	o	o	o	o	o
54	+	+	+	+	+	+	+	o						
55	+	+	+	+	+	+	+	o						
56	+	+	+	+	+	+	+	o+						
66	-	-	-	-	-	-	-	o	o	o	o	o	o	o
67														
68	-	-	-	-	-	-	-	o	o	o	o	o	o	o
71														
72	+	+	+	+	+	+	+	+						
74														
75	+	+	+	+	+	+	+	o						
77	+	+	+	+	+	+	+	o						
79	-	-	-	-	-	-	-	o	o	o	o	o	o	o
81	+	+	+	+	+	+	+	o						
102														
103	+	+	+	+	+	+	+	o						
104	-	-	-	-	-	-	-	o	o	o	o	o	o	o
109	+	+	+	+	+	+	+	o						
110	+	+	+	+	+	+	+	o						
111	+	+	+	+	+	+	+	o+						
CONTROLS														
A	+	+	+	+	+	+	+							
B	+	+	+	+	+	+	+							
C	+	+	+	+	+	+	+							
D	+	+	+	+	+	+	+							
E	+	+	+	+	+	+	+							
F	+	+	+	+	+	+	+							
G	+	+	+	+	+	+	+							

APPENDIX IV.

Bacterial Examination of Slaughterhouse Blood during Preparation of IgM Fraction.

Date	Blood col/ml.	Serum col/ml.	Euglobulin col/ml.	Organisms.
13.10.70	Nil	Nil	150	Staphylococcus
20.10.70	060	008	150	"
28.10.70	160	100	040	"
3.11.70	300	800	1,050	Staphs. + E.coli
10.11.70	440	400	20	Staphs. + E.coli
17.11.70	1,000	1,000	4,000	Proteus + E.coli
24.11.70	32	80	60	Staphs. + E.coli
1.12.70	32	4	4	Staphs.
8.12.70	16	16	Nil	E.coli
15.12.70	40	28	4	E.coli
22.12.70	8	Nil	Nil	E.coli
5. 1.71	1,000	1,000	Nil	E.coli
12. 1.71	Nil	20	4	Staphs.
19. 1.71	8	8	240	Staphs.
26. 1.71	Nil	Nil	Nil	-
16. 2.71	280	500	40	Staphs. + E.coli
23. 2.71	8	24	60	Staphs.
2. 3.71	60	160	100	Mixed + E.coli
9. 3.71	300	16	Nil	Staphs. + E.coli
16. 3.71	300	300	200	Mixed
23. 3.71	20	16	100	Staphs.
30. 3.71	20	12	24	E.coli
6. 4.71	4	Nil	Nil	E.coli
13. 4.71	Nil	Nil	Nil	-

APPENDIX V

SUMMARY OF BACTERIOLOGICAL FINDINGS IN CALVES RECEIVING IgM FRACTION INTRAVENOUSLY

Daily Examination of Peripheral Blood

CALF	0	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
SINGLE DOSE																													
106	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+1	+3	+2	+2	+3	+3								
107	-	-	-	-	-	-	-	-	-	-	+4																		
77	-	-	-	-	-	-	-	-	-																				
112	-	-	-	-	-	-	-	-	-	-	-	-	+1	+4															
113	-	-	-	-	-	-	-	+1	+2	+3																			
125	-	-	-	-	-	-	-	-	-	-	-	+2	+1	+2	+2	+2	+2	+4	+4										
DOUBLE DOSE																													
117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
118	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
119	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+2	-	-	-	-	-	-	-	-
122	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
124	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
136	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
140	-	-	-	-																									
141	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CONTROLS																													
1	-	+4																											
2	-	-	+3	+4																									
3	-	+3	+4																										
4	-	-	+2	+2	+3																								

Post Mortem Examination

CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	STIFLE JOINT	SMALL INTESTINE	MESENTERIC LYMPH NODES					
									1	2	3	4	5	6
SINGLE DOSE														
106	+	+	+	+	+	+	+	o						
107	+	+	+	+	+	+	+	o						
77	-	+	-	+	-	-	-	o						
112	+	+	+	+	+	+	+	o						
113	-	+	+	+	+	-	-	o						
125	+	+	+	+	+	+	+	o						
DOUBLE DOSE														
117														
118														
119														
121														
122														
123														
124	-	-	-	-	-	-	-	o	o	o	o	o	o	o
136														
138														
139														
140	-	-	-	-	-	-	-	o	o	o	o	o	o	o
141														
CONTROLS														
1	+	+	+	+	+	+	+	+						
2	+	+	+	+	+	+	+	o						
3	+	+	+	+	+	+	+	+						
4	+	+	+	+	+	+	+	o						

+ = <u>E. Coli</u> 078K80(B)

o = <u>E. Coli</u> NOT 078K80(B)

1 = 1 - 10 Colonies <u>E. Coli</u> 078K80(B)/ml

2 = 10 - 100 Colonies <u>E. Coli</u> 078K80(B) "

3 = 100 - 1000 Colonies <u>E. Coli</u> 078K80(B) "

4 = > 10,000 Colonies <u>E. Coli</u> 078K80(B) "

APPENDIX VI

SUMMARY OF BACTERIOLOGICAL FINDINGS IN MARKET CALVES

Daily Examination of Peripheral Blood

CALF	0 AM	0 PM	1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM
CONTROLS																														
99	-	-	o	o																										
193	-	-	-	-	+	+																								
537	-	-	-	-	-	-	-	-	-																					
195	-	-	-	-	-	-	-	-	-	-	o																			
107	-	-	-	-	-	-	-	-	-	-	-	-	-																	
69	-	-	-	-	-	-	-	-	-																					
67	-	-	-	-	-	-	o																							
IgM^{I}/V																														
M94	-	-	-	-	-	-	-	-	-	-																				
J92	-	-	-	-	-	-	-	-																						
M81	-	-	-	-	-	-	-	-																						
M197	-	-	-	-	-	-	-	-																						
M95	-	-	-	-	-	-	-	-	-																					
M109	-	-	-	-	-	-	-	-	-	-	-	-																		
148	-	-	-	-	-	-	-	-	-																					
M185	-	-	-	-	-																									
189	-	-	-	-	-	-	-	-	-																					
M191	-	-	-	-	-	-	-	-	-	-	-	-	-	-																
IgM + WHEY																														
166	-	-	-	-	-	-	-																							
162	-	-	-	-	-	-	-	-	-	-	-	-	-																	
167	-	-	-	-	-	-	-	-	-	-	-	-																		
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
175	-	-	-	-	-	-	-	-	-	-	-	-	-	-																
WHEY																														
165	-	-	-	-	-	o																								
181	-	-	-	-	-	-																								
163	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	o	o								

Post Mortem Examination

CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	STIFLE JOINT	SMALL INTESTINE	MESENTERIC LYMPH NODES 1	2	3	4	5	6
CONTROLS														
99	o	o	o	o	o	o		o	o	-	o	-	o	-
193	+	+	+	+	+	+	+	o	o	o	o	-	o	-
537	-	-	-	-	-	-	-	o	-	o	-	o	-	o
195	o	o	-	-	o	-	-	o	o	o	o	o	o	o
107	-	-	-	-	-	-	-	o	o	o	o	o	o	o
69	-	-	-	o	o	o	-	o	o	o	o	o	o	o
67	o	o	o	o	o	-	-		-	-	o	o	-	-
IgM^{I}/V														
M94	-	-	-	-	-	-	-	o	o	o	o	-	o	o
J92	-	-	-	-	-	-	-	o	o	o	o	o	o	o
M81	-	-	-	-	-	-	-	o	o	-	o	o	-	o
M197	-	-	-	-	-	-	-	o	o	o	-	o	o	o
M95	-	-	-	-	-	-	-	o	o	-	o	o	-	-
M109	-	-	-	-	-	-	-	o	o	o	o	-	o	-
148	-	-	-	-	-	-	-	o	o	o	-	o	o	o
M185	-	-	-	-	-	-	-	o	o	o	o	o	o	o
189	-	-	-	-	-	-	-	o	o	o	o	-	o	o
M191	-	-	-	-	-	-	-	o	o	o	o	o	o	o
IgM + WHEY														
166								o	o	o	o	o	-	o
162								o	o	o	o	o	o	o
167								o	o	o	o	o	o	o
180														
174														
175								o	-	o	-	-	-	-
WHEY														
165		-	o	-	o	-	-	o	NOT SAMPLED					
181		o	o	o	o	o	o	o	o	-	-	o	-	-
163		o	o	o	o	-	-	o	-	-	-	-	-	-

\+ = *E. Coli* 078K80(B)

o = *E. Coli* NOT 078K80(B)

A P P E N D I X IX

SUMMARY OF BACTERIOLOGICAL FINDINGS ON CALVES EXPERIMENTALLY CHALLENGED WITH E. COLI O101K(A?)

Daily Examination of Periphal Blood								Post Mortem Examination													
CALF	0	1	2	3	4	5	6	CALF	SPLEEN	LIVER	KIDNEY	UMBILICAL VEIN	HEART	LUNGS	SMALL INTESTINE			MESENTERIC LYMPH NODES					
															UPPER 1	MIDDLE 2	LOWER 3	1	2	3	4	5	6
131	-	-	-	-				131	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o
132	-	-	-	-	-			132	-	-	-	-	-	-	o	o	-	o	o	o	o	-	-
133	-	-	-					133	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o
135	-	-	-	-	-	-	-	135															
137	-	-	-					137	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o
145	-	-	-	-	-	-	-	145															
146	-	-	-	-				146	-	-	-	-	-	-	o	-	-	o	o	-	o	-	-
147	-	-	-	-				147	-	-	-	-	-	-	o	o	o	-	o	o	o	o	-
148	-	-	-	-	-	-		148	-	-	-	-	-	-	-	o	-	o	o	o	o	o	o
149	-	-	-					149	-	-	-	-	-	-	o	o	-	o	o	o	o	o	-
151	-	-	-	-				151	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o
152	-	-	-					152	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o
153	-	-	-	-				153	-	-	-	-	-	-	o	o	o	o	o	o	o	o	o

o = E. Coli O101K(A?)

APPENDIX VII.

When designing a restraining crate for calves and in particular newborn calves, certain factors must be taken into consideration. Firstly, between and within breeds there is wide variation in size and therefore a crate must be adjustable. Secondly, because the newborn calf is ungainly and unsteady on his legs it is essential that the crate whilst being restrictive does not impede the calf when it is attempting to rise or lie down. When rising, bovidae stand on to their hind legs first, then the forelegs. During the latter movement, the newborn calf often takes one or two steps forward in order to retain its balance and so a crate must be long enough to allow this. Thirdly, the floor must be slip proof and of such a material that it does not damage the soft horn of the calf's foot, e.g. wire mesh has a severe rasp-like effect on soft horn. Fourthly, the crate should be enclosed to make it draught proof and provide privacy for the calf allowing it to rest undisturbed by activity around it. During the present studies a crate which fulfilled most of the above criteria evolved.

Basically, (Fig. 44) it consisted of a cage which was 3'9" long, with an adjustable side (not shown) which could be moved to increase or decrease the width. There were doors, front and back, to allow easy access to the calf. The cage was enclosed with hardboard, a space being left in the rear door to facilitate the collection of faeces. The floor of the cage was perforated metal but this proved to be very slippery when wet and so a slatted teak floor was made according

FIGURE 44.

Restraining crate for calf.
Adjustable side has been removed.

to specifications recommended for calf houses. Within the narrow confines of the crate the teak slats proved unsuitable and it was necessary to cover it with strips of pyramid rubber matting. This proved to be ideal and the calves were able to rise without any difficulty.

With experience, it was possible to adjust the width of the crate to allow the calf sufficient room to lie comfortably but yet prevent it turning around. Calves require more width when lying than standing and it was found that when the crate was wide enough to allow the calf space to lie at ease, it was also wide enough to allow it to turn around. This could be prevented by making the pen narrower at the top than the bottom by angling the adjustable side.

To date, 24 calves have been put into the crate and none have suffered any injury of any kind as a result.

APPENDIX VIII

A collector was designed to fit an average Ayrshire bull calf by making a cast of the rump and perineal region of a calf. On this cast was moulded a latex rubber cone which closely followed the contours of the calf and so prevented leakage of faeces, particularly when the calf was lying down. To the end of the cone was taped a nylon sleeve in which the faeces collected.

The gluteal and perineal regions of an average Aytshire calf (35 kg. body weight) were smeared with paraffin wax; moistened strips of plaster of paris bandage (Gypsona) were applied and allowed to dry in situ. Sufficient bandage was used to form a rigid structure. When dry, the mould was placed in a tray and packed around with damp sand and the inside coated with paraffin wax. The mould was now filled up with modelling plaster in order to make the cast which was build up into a cone shape using plastic and glass containers (Fig.45). Layers of latex rubber adhesive (Dunlop Latex Co. Ltd.) were painted on the cast, each layer being allowed to dry before the application of another. After 7 - 8 coats had been applied, gauze was spread over the mould and covered with more layers of latex. The gauze added more strength and rigidity to the collector. Webbing straps with buckles were fixed to the latex with contact adhesive (Fig.46) 2 dorsally, 2 ventrally and 2 laterally. Another layer of gauze and several more layers of latex followed until there was a final thickness of $\frac{1}{8}$". When dry the latex

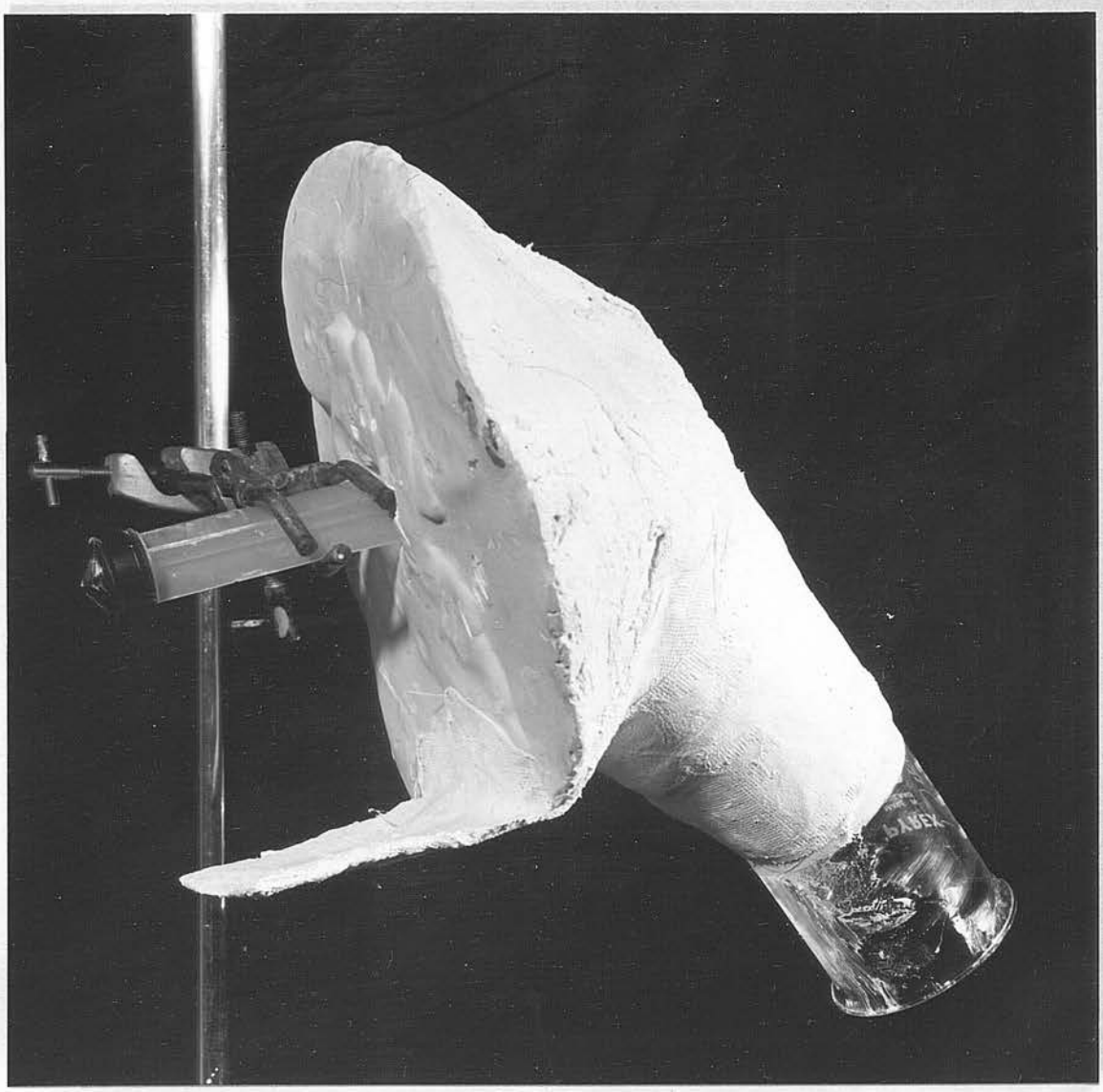

FIGURE 45

Plaster cast for faeces collector.

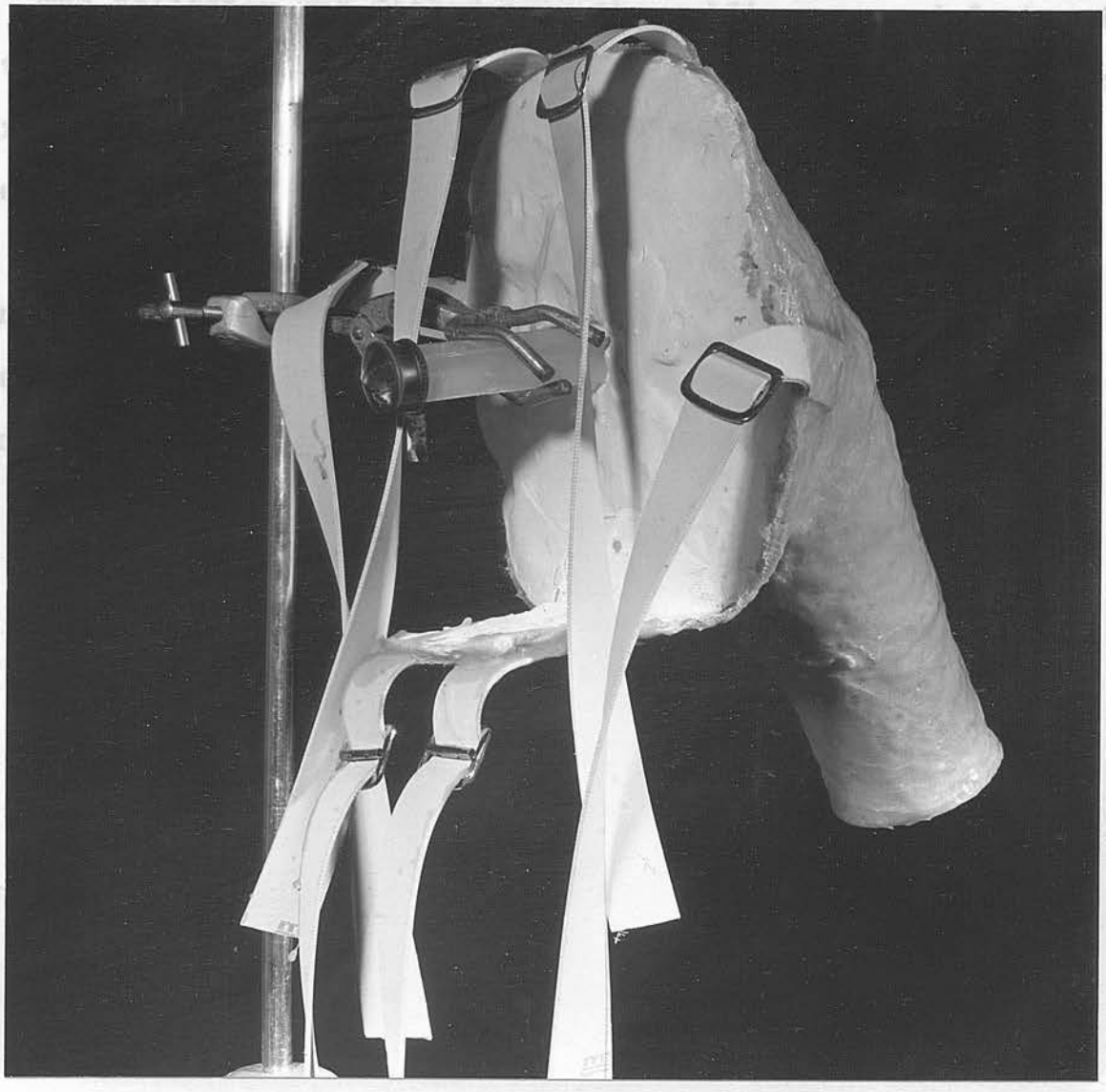

FIGURE 46

Collector with webbing straps.

was peeled from the cast.

The collector was fitted over the calf's rump and fastened by the straps to a body belt (Fig.). It was found necessary to fix the body belt to a collar around the calf's neck, otherwise it tended to slip backwards. By using latex rubber and gauze, the collector retained its shape when the calf was lying down and channelled the faeces into the nylon sleeve. It was thus possible even when calves had severe diarrhoea to collect faeces and urine separately.

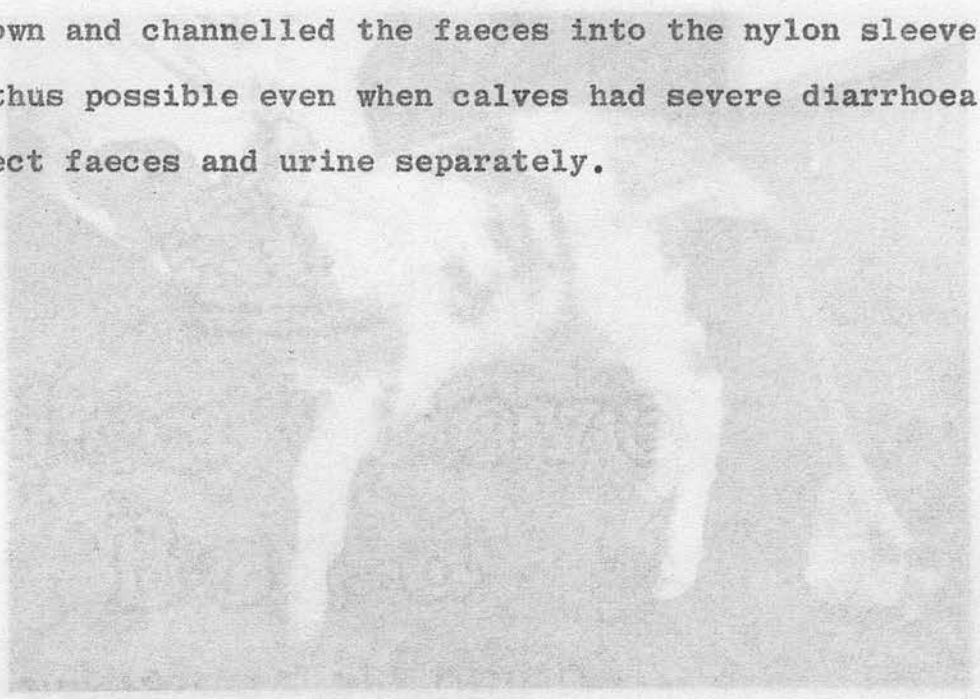

FIGURE 47

Calf showing attachment of faeces collector.

PUBLISHED PAPERS.

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Studies on the Immunity of the Calf to Colibacillosis

I. The Influence of Colostral Whey and Immunoglobulin Fractions on Experimental Colisepticaemia

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SUMMARY.—Neonatal colostrum-deprived calves were given colostral whey and immunoglobulin fractions (IgG and IgM) intraperitoneally prior to challenge with a pathogenic *E. coli* serotype (078 K80(B)). Administration of whey above a certain level, calculated in terms of its immunoglobulin content, protected calves against septicaemia, but did not inhibit enteric disease. In order to prevent septicaemia the effective protective dose (E.D.) 95 of the pool of colostrum used contained 0·26 g. IgM and 1·5 g. IgG per 30 kg. bodyweight. Colostral IgG and IgM fractions given separately at levels in excess of those in the E.D. 95 of whey both failed to prevent septicaemia. However, the IgM fraction significantly prolonged survival time ($p < 0.016$) and delayed the onset of septicaemia ($p < 0.014$) when compared with untreated control calves challenged with the same serotype.

Introduction

THE SIGNIFICANCE of colostrum, in conferring resistance on the newborn calf against infection, has long been appreciated. As early as 1905, Jensen showed that feeding colostrum protected calves from fatal "enteritis". Later, it was shown (Howe, 1924, Smith & Little, 1922) that until colostrum had been ingested, the plasma of the neonatal calf was devoid of the globulins containing the antibodies which were believed to be the protective factors. In 1949, Aschaffenburg and co-workers fed various colostral fractions to unsuckled calves and noted that only those calves receiving the globulin fraction survived and thrived. As little as 80 ml. of aqueous whey containing the immune lactoglobulin would protect calves against colibacillosis. Under natural conditions Fey and Margadant (1961) found that calves which died of colisepticaemia were deficient in plasma gammaglobulin, and subsequently, Gay *et al.* (1965) demonstrated a relationship in market calves between the clinical nature of the disease and the plasma gammaglobulin concentration using a zinc sulphate turbidity test to assess the immune globulin content. Later, the physio-chemical heterogeneity of colostral immunoglobulins was demonstrated (Murphy, Aaland, Osebold & Carroll, 1964; Pierce & Feinstein, 1965) and it is now possible to differentiate these into classes (IgA, IgG and IgM) on this basis. Although all classes have the common property of antibody activity their heterogeneity reflects differences in secondary biological activities such as their ability to fix complement. It was noted (Penhale & Christie, 1969) that quantitatively the main immunoglobulins present in bovine colostrum were IgG and IgM and that there was a fairly wide variation in the quantity of both components in different samples. In the light of these qualitative and quantitative variations it was decided to investigate the protective activity of colostrum more precisely by its immunoglobulin content.

In a study of the plasma immunoglobulin level of market calves, in relation to neonatal disease, it was found that those which developed septicaemia were deficient in both IgM and IgG, while calves with high levels survived (Penhale *et al.*, 1970). Although it was not possible from this investigation to assess the value of the individual immunoglobulin components independently, it was suggested on the basis of its natural antibody activity against *E. coli* that IgM may be the principal immune component as far as protection against colisepticaemia in the calf is concerned. In order to investigate this possibility further, the value of these immunoglobulins, isolated in relative purity from colostrum, was assessed independently in quantities related to the effective prophylactic dose (ED 95) of that particular immunoglobulin determined from whole colostral whey treatment.

Materials and Methods

Calves

Newborn unsuckled calves, mainly of the Ayrshire breed were collected as soon as possible after birth from three farms. They were weighed and placed in freshly-disinfected, individual pens. Pulse, rectal temperature and respiratory rates were recorded twice daily. At the same times, blood samples were aseptically obtained from the jugular veins, and faeces samples, taken from the rectum, were homogenised and dried to constant weight. Calves were considered to be diarrhoeic if their faecal dry matter content was less than 10 per cent.

Packed cell volume was measured by the micro-haematocrit method twice daily.

The initial serum sample was checked for the presence of immunoglobulins by immunoelectrophoresis and the single radial diffusion method.

The calves were injected intraperitoneally with the appropriate quantity of colostrum of immuno-

globulin fraction, at the left sublumbar fossa. Two hours later they were given the bacterial culture orally, using a syringe and cannula, and untreated control calves received only this culture. Calves were fed by teat milk from cows in late lactation, a maximum of 1·5 litres per 30 kg. bodyweight twice daily, and no attempt was made to feed calves which were disinclined to suckle. Milk was not fed until after the colostral whey or fractions and the challenge organism had been administered.

Post-mortem examination was carried out as soon after death as was practicable, and on occasions, when there was more than a few hours delay, the cadavers were kept in cold storage.

Sterile swab samples were taken from a number of sites, including the intestine, for bacteriological examination.

Preparation of Colostral Whey

Colostrum, taken at the first *post-partum* milking, was collected from several farms. The fat was removed by centrifugation at 820 *g* for one hour, followed by filtration through a coarse nylon filter. Casein was clotted by the addition of essence of rennet at the rate of 4 ml. per litre of colostrum. After clot retraction, the various wheys were pooled, centrifuged at 35,000 *g* for one hour and the supernatant dialysed against buffered physiological saline to pH 7·3, filtered through cellulose membranes* of diminishing pore size until it was finally sterilised bacteriologically by passing through a 0·22μ membrane. A sample was retained for analysis, and the remainder was stored at —20° C. in aliquots of 100 mls.

Preparation of Immunoglobulin M

Colostral IgM was prepared in quantities suitable for injection by diluting filtered whey with 14 volumes of distilled water. After 24 hours, the precipitate was recovered and dissolved in a suitable quantity of 0·1M. tris HCl (pH 7·9) containing 1M NaCl (approx. 20 vols. of buffer for each litre of original whey). The solution was centrifuged at high speed and the supernatant was fractionated on a 90 cm. × 64 sq.cm. column of Sephadex G 200.† To reduce contamination with other serum components, only the first half of the exclusion peak was collected, dialysed against distilled water for 24 hours to remove the buffer, concentrated by ultra filtration and freeze-dried. It was then redissolved in sterile phosphate buffered saline *p*H 7·5.

Preparation of Immunoglobulin G

Colostrol IgG was prepared by a batch DEAE cellulose procedure.

Crude IgG was obtained by precipitation from colostral whey using 28 per cent. (w/v) sodium sulphate After separation the precipitate was redissolved in 0·15M NaCl to a final volume of one quarter of that of the starting whey and dialysed overnight against a large volume (50 to 100 vols.) of phosphate buffer (pH 7·5, 0·1M.).

*Millipore Ltd.
†Pharmacia Ltd.

DEAE cellulose* was equilibrated with phosphate buffer (pH 7·5 0·1M) and used at approximately 1,000 g. wet weight to treat the globulin obtained from 1 litre of starting whey. The crude globulin was added to the exchanger and stirred for approximately 10 min. at room temperature. Phosphate buffer was then added to the thick suspension and the mixture was stirred for a further 15 min. (1 litre of buffer for 100 g. exchanger). The resultant slurry was filtered on a Buchner funnel. The filtrate was concentrated by ultra-filtration and dialysed against distilled water (1:50 vols. approx.), and finally lyophilized.

ESCHERICHIA COLI *Culture*

E. coli Serotype 078 K80(B) was used throughout the present experiments. Stock cultures were maintained by transfer on Dorset egg slopes. Weekly subculture to MacConkey agar plates was carried out and after overnight incubation these were stored at 4° C. When a calf became available growth from a smooth colony was subcultured to glucose broth and incubated for six hours. Each calf received 1 ml. per 10 kg. bodyweight of this broth which contained approximately 0·5 to 2 × 10^9 organisms per ml.

Virulence of the organism was maintained by regular re-isolation from the experimental calves which died of septicaemia, and virulence was checked by periodic use on untreated control calves during the course of the experiment.

Bacteriological Studies

A quantity of 0·25 ml. of freshly-obtained heparinised blood was spread directly on to the surface of MacConkey and sheep blood agar plates, which were incubated overnight. A number of individual *E. coli* colonies (approx 10 per plate) were tested by slide agglutination with standard antisera against 078 K80(B) prepared in rabbits (Penhale, 1965). Cultures of swabs taken from organs *post mortem* were similarly examined.

Immunoelectrophoresis and Quantitative Immunoglobulin Determination

These techniques were carried out as previously described (Penhale & Christie, 1969).

Haemagglutination Test

The preparation of *E. coli* antigens, sensitisation of erythrocytes and the haemagglutination test procedure were as described previously (Penhale, 1965).

Derivation of Estimated Protective Dose 50 *and* 95 *of Colostral Whey* (*ED* 50 *and ED* 95)

Initially, it had been decided to give the whey intravenously, but the colostrum was found, on collection from the various farms, to be heavily contaminated and, in spite of bacteriological sterilisation, caused sudden collapse and death in two calves, when given intravenously. This was presumed to be due to the presence of endotoxin or other bacterial products. The intraperitoneal route was therefore used, and although calves still showed varying degrees of shock,

*Whatman Ltd.

they recovered quite quickly, usually within two hours, at which time they were given the *E. coli* culture.

The first calf was given 250 ml. of whey per 30 kg. bodyweight which contained 1 g. IgM and 6 g. IgG. This quantity was calculated from the previous findings that market calves which survived the neonatal period had mean serum levels of IgG and IgM of 7·5 and 0·8 mg. per ml. respectively. (Penhale *et al.*, 1970).

It soon became apparent that although the parenteral administration of whey effectively excluded generalised infection, it had little influence on the diarrhoeic syndrome. It was decided, therefore, to establish a dose/effect relationship for the exclusion of septicaemia only. A calf which was not septicaemic, even though it did not survive, was considered "protected" and, using this criterion, the whey dose was adjusted up or down according to the fate of the preceding calf.

Calves were defined as septicaemic if the test organism or any other organism was isolated from the peripheral blood during life or from the organs at *post mortem* examination.

The results were examined by Probit analysis and by the method of Dixon and Mood (1948) and an ED 50 and ED 95 established for the exclusion of septicaemia.

Subsequently, calves were given IgG and IgM fractions separately, in doses in multiples of that present in the ED 95 of whey, and the results were analysed by randomisation tests.

Results

The Immunochemical and Serological Analyses of the Whey and Immunoglobulin Fractions

Examination of the immunoelectrophoretic pattern of whole whey (Fig. 1) showed that in the gamma region, in addition to the predominant component IgGl, IgA and IgM were also present. On immunoelectrophoresis (Fig. 1) the IgG preparation was found to contain a trace of IgA and the IgM preparation, a small amount of $\gamma 2$ globulin. Table I shows the relative quantities of the immunoglobulins present in the three preparations. As indicated on immunoelectrophoresis, the IgG preparation was relatively pure and no IgM was detected. However, antibody to *E. coli* O antigens as judged by haemagglutination titration was very low. The IgM preparation contained some IgG as well as other colostral proteins. There was a considerable increase in the IgM content, however, relative to that of colostral whey. The antibody activity of this fraction was also increased in comparison to the whey and in the same proportion as the concentration of IgM.

Absorption of Whey and Immunoglobulin Fractions from the Peritoneum

To assess the degree of absorption from this site pre-injection and 12 hour post-injection sera samples from the first few calves treated with whey, and from all calves treated with the immunoglobulin fractions, were examined for IgG and IgM content and also for antibody activity by haemagglutination.

TABLE I
ANALYSIS OF COLOSTRAL WHEY AND IMMUNOGLOBULIN FRACTIONS

	Total protein mg. per ml.	IgG mg. per ml.	IgM mg. per ml.	Haemagglutination Titre 09	Haemagglutination Titre 078
Colostral whey	71	24	4	128	64
IgG	10	10	—	4	4
IgM	30	2	21	2,048	1,024

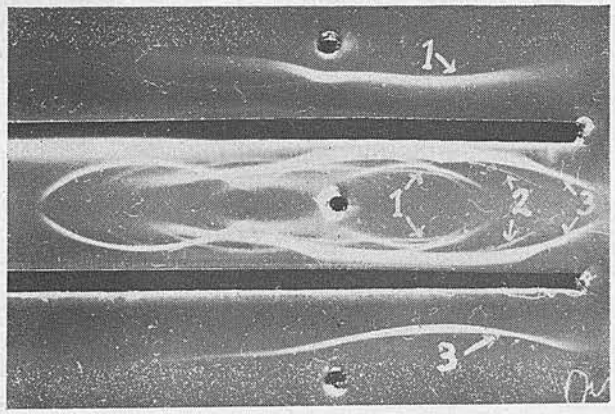

FIG. 1.—Immunoelectrophoresis of colostral whey and immunoglobulin preparations. Upper well; IgM preparation. Middle well; colostral whey. Lower well; IgG preparation. Upper and lower troughs contain rabbit anti-bovine serum.
1. IgM arcs. 2. IgA arcs. 3. IgG arcs.

Table II shows that in every case there was a post-injection increase of immunoglobulin levels indicating that effective absorption of both immunoglobulins occurred from this site. This was further supported by the observation that in both the whey and IgM-injected calves there was also a significant rise in the haemagglutinin titre.

The Effect of Colostral Whey Administration

The results of the administration intraperitoneally of colostral whey in various quantities to 36 calves are summarised in Fig. 2 in which clinical findings are related to the IgG and M content of the whey dose administered. It was observed that, clinically, treated calves could be divided into three groups:

1. Septicaemic calves which invariably died.
2. Calves which died without septicaemia and in which diarrhoea was the most prominent feature.
3. Surviving calves.

Calves which died of septicaemia received the

TABLE II
ABSORPTION OF WHEY AND IMMUNOGLOBULIN FRACTIONS FROM THE PERITONEUM

Calf No.	Dose g. per 30 kg. bodyweight		Pre-injection serum levels			12 hr. post injection serum levels		
			mg. per ml.		*HA titre 078	mg. per ml.		*HA titre 078
	IgG	IgM	IgG	IgM		IgG	IgM	
8	3·0	0·5	0·35	0	0	3·1	0·43	16
9	1·5	0·25	0·66	0	0	1·6	0	8
10	0·95	0·125	0	0	0	0·35	0	8
12	2·7	0·45	0	0	0	0·24	0	4
69	0	0·52	0	0·17	4	0	0·49	16
70	0	0·52	0	0	4	0	0·44	16
75	0	1·0	0	0	0	0	0·42	8
82	0	0·7	0	0	0	0	0·59	32
63	1·8	0	0	0	0	0·4	0	0
64	3·6	0	0	0	0	1·45	0	0
65	3·6	0	0	0	0	1·1	0	0
93	2·7	0	0·12	0	0	1·0	0	0
84	4·8	0	0	0	0	2·0	0	0

*HA = Haemagglutination test.

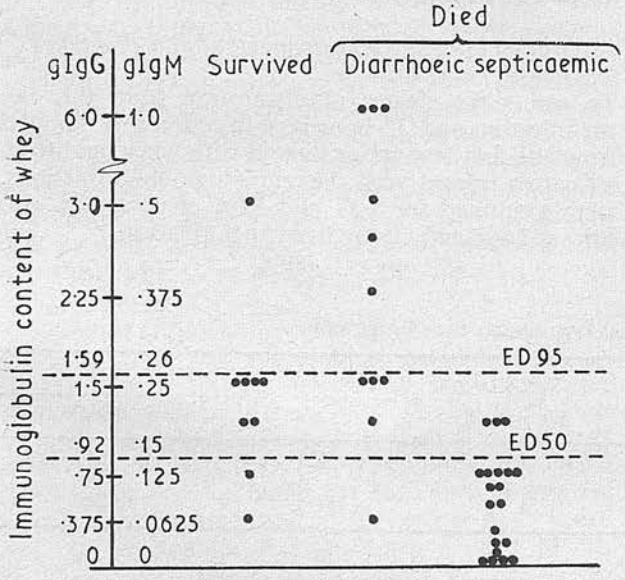

FIG. 2.—Dose-syndrome relationship in colostral whey-treated calves. Each point represents the quantity of whey given to an individual calf.

lowest doses of whey. Under the present experimental conditions, in order to exclude septicaemia, it was found that the ED 50/30 kg. bodyweight was a volume of whey containing 0·15 g. IgM and 0·98 g. IgG and for this particular sample was 37·5 ml. and the ED 95/30 kg. bodyweight was 0·26 g. IgM and 1·5 g. IgG (65 ml. whey).

Septicaemic calves received quantities of whey containing less than 0·2 g. IgM and 1·25 g. IgG (Fig. 2). Within the group no relationship was found between the survival time and the dose of whey, e.g. one calf given only 0·04 g. IgM, 0·25 g. IgG (10 ml.) survived 10 days whilst one given 0·2 g. IgM and 0·9 g. IgG (40 ml.) lived only three days.

Clinically, 12 to 36 hours after challenge, there was fever, dullness and inappetence which lasted approximately 12 hours. This was followed by a variable period of improvement before the onset of septicaemia when the calves again became febrile, increasingly weak, disinclined to feed, and eventually recumbent, after which death rapidly followed. During the initial period of depression there was invariably a severe watery, sometimes haemorrhagic, but transient, post-meconial scour, after which faeces became firmer. Diarrhoea again usually developed terminally, but this varied considerably in severity in individual calves (Fig. 3). All calves showed an increased packed cell volume during illness.

Unlike untreated calves (*vide infra*) which rapidly became septicaemic after challenge, *E. coli* was only isolated from the peripheral blood within the last 48 hours of illness except in two calves (Fig. 3). In the case of four other calves, positive blood cultures were not obtained, even though the challenge organism was isolated from organs at *post mortem*, and in two further calves, an *E. coli* serotype other than the challenge organism was isolated from the peripheral blood and organs (Table IV).

Calves which died without becoming septicaemic, received doses of whey comparable to those given to calves which survived. Clinically, the outstanding feature of these calves was the occurrence of a profuse, watery diarrhoea which commenced immediately after passage of the meconium and continued without remission until death. Survival time was extremely short, calves with one exception dying within 3·5 days which was less than the mean survival time of septicaemic calves (Table III). After an initial pyrexia, seen in all calves approximately 24 hours after challenge, a raised temperature was not noted even terminally. In contrast to the septicaemic calves, they showed a willingness to feed until just before death. In all cases, signs of severe dehydration and marked haemoconcentration were evident for a considerable time before death.

At *post mortem* examination, dehydration was a constant feature. Intestinal changes were variable, but in contrast to septicaemic calves there was an absence of congestion in the liver, lungs, kidney

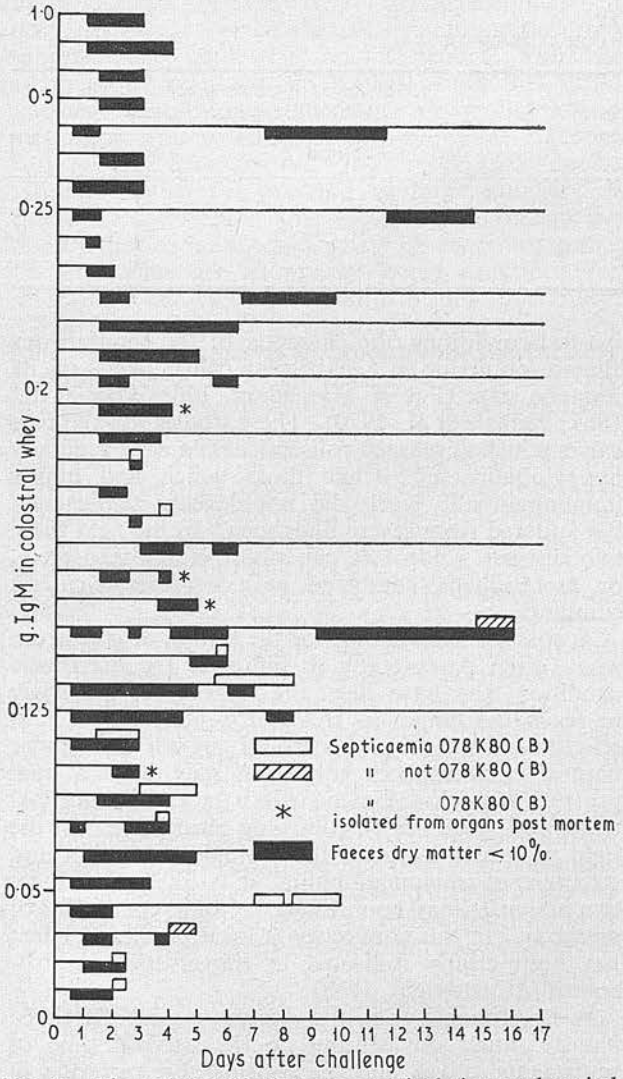

FIG. 3.—The relationship between survival time and period of septicaemia and scour in colostral whey-treated calves. The solid line indicates survival time.

and spleen. On bacteriological examination no organisms were isolated from any tissue except the mesenteric lymph nodes where variable numbers of mucoid types of *E. coli* were found. Throughout the proximal small intestine of these calves, *E. coli* generally of the mucoid type were isolated in large numbers.

Surviving Calves

Surviving calves showed some similar clinical features to those in Group I despite the absence of septicaemia. There was initial dullness and diarrhoea followed by rapid recovery. In some calves there was a second period of pyrexia at five to eight days together with diarrhoea (Fig. 3) from which calves recovered in a few days and at 14 days were thriving. At one month, these calves were slaughtered and no abnormalities were observed at *post mortem* examination. On bacteriological examination all tissues were sterile.

The Effect of IgM Administration

Four calves were given IgM before challenge in quantities in excess of that found in the ED 95 of whey. Although these calves died of septicaemia, the administration of IgM produced an obvious modification in the septicaemic pattern observed in untreated calves. The IgM significantly prolonged survival time ($p<0{\cdot}016$) and also delayed the onset of septicaemia ($p<0{\cdot}014$) compared with untreated calves and similarly with calves given IgG ($p<0{\cdot}014$ and $p<0{\cdot}016$ respectively). In these calves bacteraemia was only seen for a short period immediately before death (12 hours) and appeared to be of a different character from that of the untreated calves, there being the sudden influx of large numbers of organisms (approx. 10,000 per ml.), as opposed to the more gradual increase in the former group. This bacteraemia was accompanied by severe shock, the calves' condition rapidly deteriorating within a very short period. This syndrome was seen in all these calves. Haemoconcentration was variable. Evidence of septicaemia was also observed at *post mortem* examination and the experimental strain was isolated from every calf.

The Effect of IgG Treatment

Five calves were given doses of IgG similar to, and multiples of, that present in the ED 95 of whey (Fig. 4). As far as clinical signs were concerned these calves most closely resembled the untreated control group in that they developed septicaemia within a short period after challenge. However, although survival time was similar in both groups, the onset of septicaemia was slightly delayed in IgG-treated calves ($p<0{\cdot}048$) (Table III). The challenge organism was isolated from the peripheral blood and organs in all cases (Table 4).

TABLE III
SURVIVAL TIME OF TREATED AND UNTREATED CALVES

Treatment	Group	No. of calves	Mean survival time (days)	Range (days)
Colostral whey	Survivors	9	All surviving at three weeks	
	Septicaemic	16	5·4	2·5—16
	Diarrhoeic	11	3·0	1·5—5
IgG	Septicaemic	5	3·4	2—5
IgM	Septicaemic	4	4·8	4—5
Untreated	Septicaemic	4	2·1	1—3

TABLE IV
SUMMARY OF BACTERIOLOGICAL FINDINGS

Treatment	Total no. of calves	No. of calves septicaemic	No. of calves in which specific strain (078K80B) isolated from	
			Peripheral blood	Organs
Whey	36	16	10	14
IgG	5	5	5	5
IgM	4	4	4	4
Untreated	4	4	4	4

Untreated Calves

The experimental serotype was administered to four calves which did not receive immunoglobulins (Fig. 4). These showed the typical picture of the disease observed in a large number of 078 K80(B)-challenged calves in previous experiments. (Penhale, 1965; Logan & Penhale, unpublished observations).

In contrast to the whey-treated calves they rapidly become septicaemic (Fig. 4) and death occurred in all cases within 72 hours (Table III). At *post mortem* examination typical septicaemic changes were observed and the challenge organism was isolated from all sites (Table IV).

Discussion

The administration of whey parenterally, at a suitable level, clearly divided the neonatal scour complex into two distinct entities. Fey *et al.*, (1963) using whey intravenously and intramuscularly, in doses of 50 to 100 ml. also found that it was possible to protect calves against septicaemia, but not against diarrhoea. These findings are in keeping with the observations of a number of workers that, under natural conditions, the character of the spontaneous disease occurring in a particular calf depends on its immune state (Fey & Margadant, 1961; Gay *et al.*, 1965; Penhale *et al.*, 1970). These studies showed that calves which developed colisepticaemia were generally hypoglobulinaemic, while those which had higher immunoglobulin levels did not develop septicaemia but suffered from severe diarrhoea. In the field these two distinct syndromes can occur either separately, or more likely, combined as a septicaemic-enteric complex.

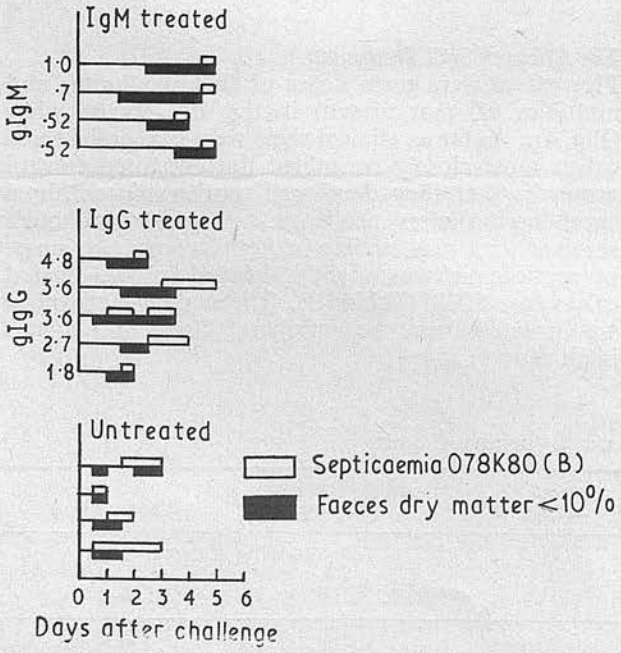

FIG. 4.—The relationship between survival time and periods of septicaemia and scour in calves receiving immunoglobulin fractions and untreated calves. The solid line indicates survival time.

A possible explanation for the failure of colostrum when given parenterally to influence the diarrhoeic condition may have been the inability of the whey to reach the lumen of the gastro-intestinal tract in effective quantities. This would suggest that under natural circumstances colostrum may have a dual protective function; locally, within the gastro-intestinal tract, and systemically, following absorption. In the light of recent work on the biological function and structure of immunoglobulins, it is likely that these two activities may be mediated by different classes of antibody. In this connection a local protective effect has been clearly indicated in transmissible gastro-enteritis (Cartwright, 1968).

It is probable that the occurrence of diarrhoea has an important bearing on the survival time of septicaemic calves, and the considerable variation in severity observed may account for the inability to establish a dose/survival time-relationship. This follows from the evidence of Marsh, Melus and Underdahl (1969) indicating that there is a loss of plasma immunoglobulins into the intestine during scouring. Depending upon the severity, there is likely to be a variable loss of circulating immunoglobulins therefore which will reduce the survival period accordingly. Studies on circulating IgG in the calf (MacDougall & Mulligan, 1969) showing that during scouring, the half life is significantly reduced, add further evidence to this possibility, and, although IgM catabolism was not investigated, it is possible that it is similarly affected.

In view of the acuteneness of the condition of the diarrhoeic, non-septicaemic calves and the fact that dehydration alone did not seem entirely to account for the marked deterioration observed, this syndrome bears some similarities to that described as enterotoxaemia by Gay, McKay and Barnum (1964). In many ways, clinically, these resembled the untreated septicaemic calves, although organisms could not be isolated from the blood or tissues *post mortem*. The marked haemoconcentration, a feature of these calves, also suggests that they might be influenced

by endotoxin derived from the gastro-intestinal tract. It would appear that this syndrome occurred spontaneously and was not associated with the administered serotype 078 K80(B), since this strain was never isolated in these cases from any tissues and was rarely found in the gastro-intestinal tract at *post mortem* examination. It is more likely to be associated with the presence of large numbers of *E. coli* of the mucoid type which were found in the small intestine and which may be enterotoxigenic. These isolates are at present being examined for enterotoxic activity to investigate this possibility.

The failure of IgG to influence to any extent the course of the disease is in accord with the inability *in vitro* to detect more than trace amounts of antibody to any *E. coli* antigens in this class of immunoglobulin by the methods employed.

In view of its *in vitro* activity against *E. coli*, the failure of the IgM fraction in concentration similar to that contained in the protective volume of colostrum to prevent septicaemia was unexpected, and may be due to to a number of factors:

(1) Purified IgM may be more slowly absorbed from the peritoneum than the IgM in natural whey, which may contain factors which enhance absorption. As a consequence, adequate levels of IgM may not be present in the circulation at the time when the invasion of the tissues by *E. coli* occurs. In support of this possibility Smith and Halls (1968) have shown that the particular serotype used in these experiments could be demonstrated in the tissues of colostrum-deprived calves within two-and-a-half hours of challenge. Once in the tissues the organisms may be protected from the effect of IgM antibodies and may later re-enter the circulation when the IgM level has fallen.

(2) During preparation, partial denaturation of the IgM component may occur, even though biological activity appeared to be retained as judged by serological tests. This could lead to a more rapid clearance than that which occurs with untreated IgM in the whey.

(3) Antibodies to *E. coli* in different immunoglobulin classes may have a combined activity which is absent from the relatively pure fractions. It is also possible that antibodies in immunoglobulin classes other than those investigated here may be responsible for protection. In this connection IgA has definitely been identified in bovine colostrum by Mach, Pahud and Isliker (1969) and this finding has been confirmed in this and other laboratories. However, present-day concepts ascribe a local protective function at the paramucosal surface to this class of immunoglobulin rather than systemic activity.

(4) A further possibility is that colostral whey may contain other protective factors in addition to immunoglobulins.

These various possibilities are at present under investigation.

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Résumé

On a donné, à des veaux nouveaux-nés privés de colostrum, du petit-lait colostral et des fractions d'anti-globuline (IgG et (gM) intrapéritonéallement avant de les soumettre à un sérotype pathogénique *E. coli* (078 K80(B)). L'administration du petit-lait au dessus d'un certain niveau calculé selon sa teneur en anti-globuline, a protégé les veaux contre la septicémie mais n'a pas empêché la maladie entérique. En vue d'éviter la septicémie, la dose protectrice effective (E.D.) 95 de colostrum utilisé contenait 0,26 g IgM et 1,5 g. IgG/30 Kg du poids du corps. Des fractions IgG et IgM de colostrum données séparément à nes niveaux excédant ceux de E.D. 95 du petit-lait, n'ont pas empêché la septicémie. Toutefois, la fraction IgM a prolongé le temps de survie d'une manière significative ($p < 0,016$) et retardé le début de la septicémie ($p < 0,014$) par comparaison aux sujets de contrôle non traités au même sérotype.

Zusammenfassung

Neugeborene Kälber, denen das Kolostrum versagt wurde, erhielten vor der Infektion mit einem pathogenen *E. coli* Type 078 K80 (B) eine Intraperitonealinjektion von Kolostrum-Molke und Immunoglobulinfraktionen (IgG und IgM). Die Verabfolgung von Molke mit einem Immunoglobulingehalt über einem bestimmten Niveau immunisierte die Kälber gegen Septikämie, gewährte jedoch keinen Schutz gegen Darmkrankheiten. Um Septikämie zu verhüten, enthielt die wirksame Schutzdosis 95 des verwendeten Mischkolostrums 0,26 g IgM und 1,5 IgG/30 kg. Körpergewicht. Separat in grösseren Mengen als in der wirksamen Schtutzdosis 95 verabfolgte Kolostrums-IgG-und IgM-Fraktionen bewirkten keine Immunität gegen Septikämie. Allerdings verlängerte die IgM-Fraktion, im Vergleich zu den mit demselben Stamm infizierten Kontrallkälbern, wesentlich die Uberlebenszeit ($P < 0,016$) und verzögerten den Ausbruch der Septikämie ($P < 0,014$).

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Studies on the Immunity of the Calf to Colibacillosis*

II. Preparation of an IgM-Rich Fraction† from Bovine Serum and its Prophylactic Use in Experimental Colisepticaemia

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Vet. Rec. (1971). **89.** *623-628*

SUMMARY.—A simple procedure is described for the preparation of an IgM-rich fraction from pooled bovine serum. Successive batches of this fraction contained high concentrations of IgM relative to their parent serum pools and antibodies to the O antigens of a number of calf pathogenic strains of *E. coli* were also correspondingly concentrated.

When given parenterally to neonatal calves prior to oral infection with a highly virulent septicaemic strain of *E. coli* (078K80B) it was found that even the lowest doses of the IgM preparation employed prolonged the survival time and delayed onset of septicaemia as compared to control untreated calves. At higher levels complete prophylaxis was achieved but this effect was not consistent and it was not possible to establish a dose/survival time relationship.

All pre-treated calves developed diarrhoea of varying severity indicating that parenterally administered IgM has little influence on the enteric situation. Nevertheless the onset of scouring appeared to be slightly delayed by this procedure.

Introduction

SINCE Smith and Little (1922) first suggested that bovine serum might be used as an alternative to colostrum to protect the newborn calf against colibacillosis, many investigations have been made in which a variety of blood-derived products, such as citrated whole blood, normal and hyperimmune sera, and serum fractions have been used for this purpose (Dollahite, 1939; Wise & Anderson, 1944; Anderson, Dupre & Lamaster, 1952; Christian and Segard, 1953; McDonald & Oakley, 1961; Fey, Margadant, Nicolet & Hunyady, 1963; Lotan, Berman, Tadmor & Perk, 1964; Watt, 1967; Urban, 1968). These studies, however, have yielded only equivocal evidence on the value of seroprophylaxis in this disease. Nevertheless, there is considerable evidence that the bulk of the immune constituents largely responsible for the protective action of colostrum, are derived unmodified from the blood (Askonas, Campbell, Humphrey & Work, 1954; Larson, 1958; Feldman, 1961).

The major immune component present in normal bovine serum having antibacterial activity, particularly against Gram negative bacteria including pathogenic serotypes of *E. coli*, is Immunoglobulin M (IgM) (Penhale, 1965a & b). This is in accordance with similar findings in other species where most natural antibodies against a variety of Gram negative organisms have been found in this particular class of immunoglobulin (Michael, Whitby & Landy, 1962; Pike & Schulze, 1964; Cohen & Norins, 1968). Furthermore, experiments with *Salmonella typhimurium* indicated that IgM antibodies are more efficient in complement-mediated bacteriolysis and as opsonins than IgG antibodies (Robbins, Kenny & Suter, 1965) and horse IgM pneumococcal antibodies have been shown to be some 100,000 times more effective than IgG in mouse protection experiments (Hill & Robbins, 1966). IgM has also been shown to be present in relatively large quantity in bovine colostrum where it is generally found at higher concentration than in serum (Penhale & Christie, 1969) and to contain specific antibodies to pathogenic serotypes of *E. coli* (Logan & Penhale, 1971). For these reasons this component has been considered to play the major part in conveying passive immunity to Gram negative infection to the newborn calf (Penhale, 1965b; Klaus, Bennet & Jones, 1969; Penhale, McEwan, Selman & Fisher, 1970).

This study describes a simple procedure for obtaining an IgM-rich fraction from normal bovine serum

* Part I of this series of papers was published in THE VETERINARY RECORD on February 27th, 1971, page 222.

† The commercial use of this preparation is covered by patent No. 1210063.

and of the assessment of its value as a prophylactic measure in experimental colisepticaemia. The evidence presented indicates that this fraction, irrespective of dosage employed in this study, had a considerable modifying effect on the usual course of the disease, although it frequently failed to prevent the ultimate onset of septicaemia, particularly at the lower dose levels. However, it had little effect upon the development of enteric disease. These findings underline the complex nature, both of the disease problem and of colostrum-derived immunity.

Materials and Methods

Preparation of IgM-rich Fraction

The method employed for IgM concentration from serum is summarised in Fig. 1.

The starting material was pooled bovine blood collected at the abattoir as aseptically as practicable. The blood was defibrinated and the serum then separated by centrifugation. The IgM rich fraction, obtained by precipitation of the euglobulins by dilution in deionized distilled water (1:14) was harvested after 48 hours by centrifugation in a continuous flow rotor (M.S.E.). Bacteriological sterilization was achieved by filtration on a cellulose membrane with 0·22μ average pore diameter (Sartorius). After the final stage; redissolving in PBS at 10 per cent. the material was stored in aliquots of 30 ml. and a sample from each batch was assayed by a number of procedures including the single radial diffusion method (SRD).

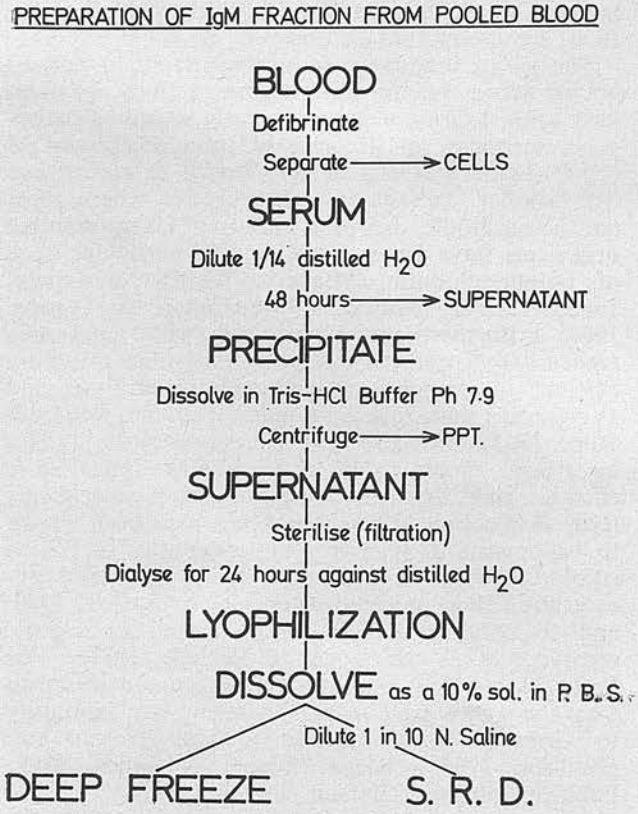

FIG. 1.—Flow diagram showing preparation of IgM-rich fraction.

Calves. Colostrum-deprived newborn calves obtained from a single source and managed as described previously (Logan & Penhale, 1971) were given varying doses of IgM fraction intraperitoneally and infected orally two hours later with the experimental *E. coli* serotype. It was found necessary to administer the IgM fraction intraperitoneally because it caused shock when given in high concentrations by the intravenous route. This toxicity was assumed to be largely the consequence of bacterial contamination. It was however, well tolerated by the intraperitoneal route causing only mild depression which generally passed within one to two hours.

Each calf was examined twice daily and jugular blood samples were obtained by aseptic technique and examined for the presence of organisms by culture.

Escherichia coli. As in previous experiments (Logan & Penhale, 1971) a highly virulent strain of serotype 078K80(b) was used throughout and maintained and administered by the same procedures.

Bacteriology. The daily blood samples and swabs taken from various organs of calves which died were examined bacteriologically for the presence of organisms, particularly *E. coli* of the experimental strain by the methods previously described (Penhale, 1965a; Logan & Penhale, 1971).

Immunoelectrophoreses and Quantitative Immunoglobulin Determination. The micro immunoelectrophoresis method of Scheidegger (1955) using barbitone acetate buffer, pH 8·6, 0·1 ionic strength (Oxoid Ltd.) was used to investigate the protein composition of the IgM preparations. Conventional electrophoresis was also carried out on cellulose polyacetate strips (Sepraphore III, Gelman Instrument Co.) with 0·05M sodium barbital-barbituric acid tris buffer (pH 8·8). Quantitative immunoglobulin determinations of IgG and IgM were made by a slight modification of the method described by Mancini, Carbonara and Heremans (1965) (Penhale & Christie, 1969).

Measurement of Total Protein. This was carried out by the Buiret method.

Gel Filtration Chromatography. Adult serum and IgM-rich preparations were fractionated on 60 × 4·4 cm. columns of G200 Sephadex (Pharmacia Ltd.) using 0·1M Tris HCl buffer (pH 8) containing 1M NaCl.

Haemagglutination Test. O antigen extracts of a number of pathogenic strains of *E. coli* were prepared and treated for use in the haemagglutination test as previously described (Penhale, 1965a).

Results

Analysis of IgM Fractions. Successive batches of the IgM-rich fraction prepared by precipitation with distilled water were compared with their respective parent serum pools by a number of analytical methods.

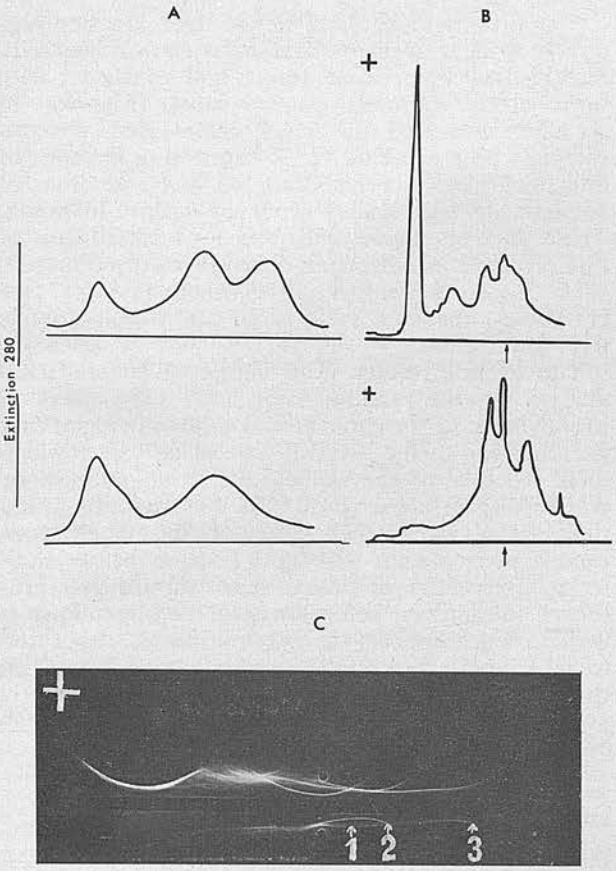

FIG. 2.—(a) Sephadex G 200 profiles of pooled adult serum (upper) and IgM rich fraction (lower); (b) Electrophoretic profile of pooled adult serum (upper) and IgM rich fraction (lower); (c) Immunoelectrophoresis of pooled adult serum (upper well) and IgM rich fraction (lower well) against rabbit anti-whole bovine serum (trough). 1, IgM. 2, $7\gamma_1$ (possibly a component of complement 3, IgG).

Fig. 2(a) shows the typical profile of this IgM-rich fraction obtained by chromatographic separation on a column of G200 Sephadex. Of the two peaks which are apparent, the first, composed of large molecular 19S components including IgM, is larger than the second 7S peak. This contrasts with the elution profile of serum on the same material where the 19S first peak is smaller than the second 7S peak and where an additional third 4·5S albumin-containing peak is observed. Analysis by electrophoresis on cellulose polyacetate membrane Fig. 2(b) revealed that the IgM preparation has a similarly restricted protein profile by this method as compared to the parent serum sample. In this case the electrophoretically-fast albumin peak and β components were almost entirely absent, while the gamma region, particularly the faster portion, was concentrated.

Immunoelectrophoretically, the IgM preparation was also found to be much more homogeneous than serum and only five clearly defined protein arcs mainly in the β and γ regions could be readily observed Fig. 2(c). Furthermore, the IgM arc was more prominent than in serum and albumin barely apparent, indicating a reversal of the concentrations found in the parent material. In addition to these, a γ_1 arc, thought to be a complement component was very prominent in all the IgM preparations using this technique.

Table I presents a comparison of the composition of five consecutive batches of the IgM-rich preparation compared with that of their parent serum pools. This shows that for an equivalent protein content a substantial concentration of IgM was consistently obtained and this component, invariably the predominant molecular species, represented approximately half the total protein present. In contrast, IgG was not concentrated by this procedure and was present at about the same concentration as in serum. Antibody titres to a number of *E. coli* somatic antigens including 09 and 078 were increased to a level corresponding to the degree of IgM concentration (approximately ten fold). However the yield of IgM recovered in each batch represented only a small proportion (between 5 to 10 per cent.) of the total IgM present in the starting volume of parent serum pool used.

Prophylactic Use of IgM Fraction in Experimental Colisepticaemia

Fig. 3 summarises the results observed when IgM fractions were administered prophylactically to 29 newborn colostrum-deprived calves which were subsequently infected with the experimental serotype. It was found that even very small quantities of this preparation had a marked influence on the usual course of the experimental disease as observed in untreated control calves (Fig. 4). Although complete protection was not achieved with these low levels, the effect of the fraction was evident in prolongation of the survival time and delayed onset of septicaemia. When septicaemia occurred it was invariably found to involve the experimental serotype and was either of a peracute form, where the bacteraemia rapidly increased to reach very high levels within a few hours of onset, or less acute, with a more gradual increase in the numbers of circulating bacteria taking place over a period of several days. In the former type, deterioration was very rapid and the calves died with symptoms of extreme shock within a few hours on

TABLE I
ANALYSES OF SUCCESSIVE BATCHES OF IGM-RICH FRACTIONS COMPARED WITH PARENT SERUM POOLS

	1	2	3	4	5
Serum					
Volume, litres	10	20	10	10	10
Total Prot. g.%	8·4	6·57	7·68	7·44	5·7
IgM mg. per ml.	5·9	3·9	4·7	4·8	3·05
IgG mg. per ml.	25·5	15·1	16·65	14·0	14·4
09 Titre	128	128	128	128	64
Total IgM g.	59 0	78 0	47 0	48·0	30·0
IgM Prep. 10% *Sol.*					
Volume, ml.	55·0	87·5	101·0	55·5	59·0
Total Prot. g. %	7·98	8·07	7·77	8·34	8·64
IgM mg. per ml.	49·0	37·0	42·0	43·0	50·0
IgG mg. per ml.	15 0	15 0	10·0	10·0	13 0
09 Titre	4,096	2,048	2,048	4,096	2,048
Total IgM g.	2·69	3·23	4·24	2·38	2·95

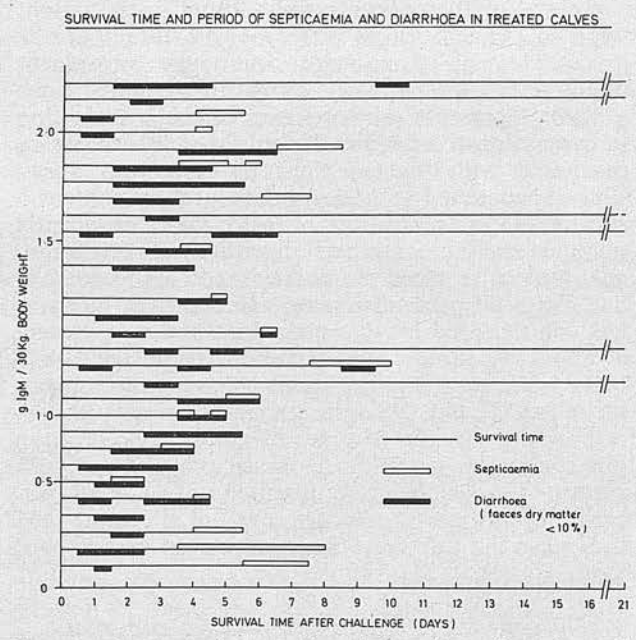

FIG. 3.—Survival time and periods of septicaemia and diarrhoea in pre-treated calves.

the onset of bacteraemia, while in the less acute form a slower but progressive deterioration took place over a period of some days.

Six treated calves died without evidence of septicaemia either by *ante-mortem* isolation of bacteria from the peripheral circulation or from the tissues *post-mortem* and in these cases the most prominent clinical feature was severe diarrhoea (Fig. 3). Although the experimental serotype was rarely isolated from the gastro-intestinal tract, in the majority of these calves mucoid strains of *E. coli* could be readily cultured from the upper intestine and in some cases also from the mesenteric lymph nodes.

When given at levels of over 1 gm. (approximately 25 ml. of IgM fraction depending on the batch) a number of calves survived and showed no evidence of septicaemia during the experimental period, although this occurred irregularly and appeared unrelated to dosage above the 1 gm. level.

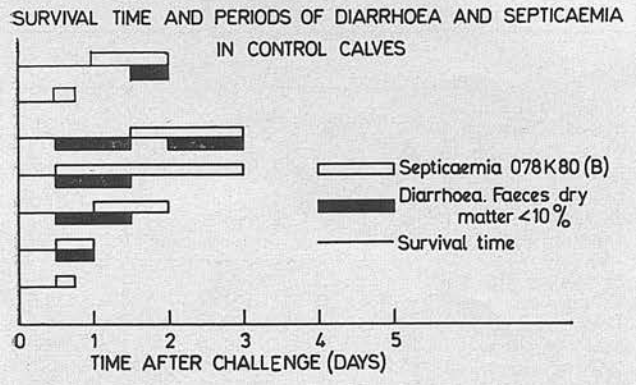

FIG. 4.—Survival time and periods of septicaemia and diarrhoea in control calves.

The prophylactic effect of the IgM fraction was clearly evident even in those individuals which ultimately died when these results are compared with those of the untreated control calves (Fig. 4). In all cases untreated challenged calves died of septicaemia within a period of 72 hours from the time of infection (mean survival time, 43 hr.) and onset of bacteraemia was always observed within 36 hours. These findings agree with previous experience of this serotype in colostrum-deprived calves (Penhale, 1965; Logan & Penhale, 1971) where in every case (15 calves) challenge resulted in death within a few days of infection.

The overall results were subjected to statistical analysis by the randomisation test. The effect of prophylactic IgM treatment was evident in significant prolongation of the survival time of all calves which died, whether of the septicaemia/scour complex or enteric disease alone ($p<0.002$). It was also found that in the calves which ultimately developed septicaemia pretreatment with IgM fraction before challenge, irrespective of dosage level, significantly prolonged the interval before onset of bacteraemia ($p<0.001$). Furthermore, the preparation also appeared to have some direct, although minor, influence on the enteric situation as pretreatment significantly prolonged the interval before onset of diarrhoea ($p<0.005$).

Discussion

Bovine blood obtained from the abattoir provides a convenient starting source of IgM as it is readily available and in virtually unlimited quantity. The method of preparation described offers a simple procedure for obtaining a suitable quantity of IgM in a convenient volume, thus permitting amounts approximating to that received by the calf under natural conditions by the ingestion of colostrum to be given by a single, small-volume injection. At the concentration which was achieved (circa 40 mg. IgM per ml.) it was found that the titre of antibody against the particular O antigens selected for use in the serological tests was similar to that which could be obtained by active hyperimmunisation. Moreover, this preparation had the advantage over hyperimmune serum that the specific antibodies of the IgM type to *all E. coli* antigens, and presumably other pathogenic micro-organisms which were present in the parent serum, were collectively concentrated, thus providing a broad spectrum of immunological activity.

The failure of the preparation to afford complete protection despite this wide biological activity was not unexpected in the light of previous experiments using more highly purified IgM of colostral origin (Logan & Penhale; 1971). It is considered that several factors may have contributed. Firstly, the toxicity of the material, which was thought to be largely due to the presence of contaminating bacterial products, in particular endotoxins, may have adversely affected the ability of the calf to withstand challenge with the highly virulent serotype of *E. coli* used. In this connection it is well-established that bacterial endotoxins

can markedly enhance susceptibility to gram negative infection (Wright, 1904; Delaunay, Sarciron & Pages, 1944; Conti, Le Cluff & Epscheder, 1961; Dubos & Schaedler, 1966). Secondly, as suggested in the previous study (Logan & Penhale, 1971) it is probable that the severe scour which developed in nearly every calf could have had an important influence on the level and duration of the systemic immunity provided by the injection of IgM fraction. This likelihood arises from recent studies in which a marked reduction in the half life of circulating IgG has been shown to occur in scouring calves (MacDougal & Mulligan, 1968) and is supported by the electrophoretic study of Marsh, Mebus & Underdahl (1969), where immunoglobulin, presumably of plasma origin, has been detected in the faeces of scouring calves. By analogy with the estimation of the half life of human IgM it is probable that the normal half life of bovine IgM is very much shorter than that of IgG and therefore likely to be more severely affected by this factor. Also, as previously suggested (Logan & Penhale, 1971), the intrusion of such an uncontrollable variable as scouring represents may well account for the inability to establish a precise protective dose using parenteral immunoglobulin in these particular studies.

As in previous studies (Logan & Penhale, 1971) mucoid strains of *E. coli* were predominantly isolated from the proximal part of the small intestine of calves which died with acute diarrhoea unassociated with septicaemia. Those strains previously isolated were found on serological identification to belong to a restricted range of serotypes, the majority of which are known to be potentially enterotoxigenic (Sojka, personal communication). In the present study serological findings were again similar and it is concluded that the spontaneous acute enteric disease observed was caused by the proliferation of mucoid enterotoxigenic strains of *E. coli* within the small intestine. These strains were presumably present in the calves' environment and must have colonised the intestines within a short time of birth.

The predominance of diarrhoea despite the administration of large amounts of parenteral immunoglobulin supports the hypothesis based on previous studies (Logan & Penhale, 1971) that systemic IgM only provides partial immunological cover which appears to be largely limited to the vascular compartment. However, the present studies revealed a small but significant influence of IgM fraction within the gastro-intestinal tract in that this preparation delayed the appearance of enteric disease.

The ability of parenteral IgM to inhibit the development of septicaemia without permanent influence on the acute enteric disease should also make this fraction a useful research tool for investigating this syndrome, without the complication of septicaemia.

Finally, this study serves to emphasise the comprehensive nature of the passive immunity provided by colostrum and suggests that it embraces a number of distinct mechanisms each adapted to confer immunological protection on a particular body compartment such as the blood, intestinal lumen and possibly also the extravascular space.

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Résumé

On décrit une procédure simple pour la préparation d'une fraction riche en IgM pour le sérum bovin. Des lots successifs de cette fraction contenaient des concentrations élevées de IgM relatives aux pools de sérum de leurs parents. Il existait également des concentrations correspondantes d'anti-corps aux antigènes O d'un nombre d'espèces pathogènes de *E. coli*.

Quand administré aux veaux nouveaux-nés par voie parentérale avant l'infection orale par une espèce de *E. coli* (078K80B) septicémique très virulente, on a trouvé que même les doses les plus faibles de la préparation IgM employée prolongeaient le temps de survie et retardient le début de la septicémie, par comparaison aux veaux non traités. A des niveaux plus élevés, on a pu obtenir une prophylaxie complète, mais cet effet n'était pas compatible et il était impossible d'établir un rapport entre la dose et le temps de survie.

Tous les sujets ayant reçu un pré-traitement ont développé une diarrhée plus ou moins grave, indiquant que le IgM administré par voie parentérale avait peu d'influence sur la situation entérique. Néanmoins ce procédé semblait retarder buelque peu le début de la diarrhée.

Zusammenfassung

In vorstehendem Bericht wird ein einfaches Verfahren zur Herstellung einer IgM-reiche Fraktion aus Sammelrinderserum beschrieben. Sukzessive Serien dieser Fraktion enthielten eine höhere IgM-Konzentration als die Sammelseren, denen sie entstammten, und dadurch waren auch die Antik öper gegen die O-Antigene mehrerer für Kalber pathogene *E. coli*-Stämme entsprechend konzentriert.

Bei einer parenteralen Verabfolgung an neugeborene Kälber, gefolgt von peroraler Infektion mit einem hochvirulenten septischen *E. coli*-Stamm (078K80B), wurde festgestellt, dass selbst die geringsten Dosen des benutzten IgM-Präparats die Überlebenszeit verlängerten und, im Vergleich zu den unbehandelten Kontrollkälbern, das Einsetzen der Sepsis verzögerten. Bei Verabfolgung grösserer Mengen wurde vollständige Prophylaxe erzielt, jedoch war diese Wirkung nicht übereinstimmend und es konnte keine direkte Beziehung zwischen der Dosis und der Überlebenszeit festgestellt werden.

Sämtliche vorbahendelten Kälber litten an Durchfall verschiedenen Grades, woraus sich schliessen lässt, dass parenteral verabfolgtes IgM wenig Einfluss auf den Darmzustand ausübt. Nichtsdestoweniger wurde allem Anschein nach der Ausbruch der Kälberruhr durch dieses Verfahren etwas verzögert.

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Studies on the Immunity of the Calf to Colibacillosis

III. The Local Protective Activity of Colostrum within the Gastro-Intestinal Tract

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SUMMARY.—Hypogammaglobulinaemic market calves, under one week old, were divided into four groups. (1) control calves. (2) calves which were given an IgM rich fraction intravenously. (3) calves which were given an IgM fraction intravenously and colostral whey orally and (4) calves which were only given colostral whey orally. They were placed collectively in premises previously contaminated by calves with colibacillosis.

All the control calves died, five with colisepticaemia, two with severe diarrhoea. The IgM fraction administered alone inhibited septicaemia in all cases, but not enteric disease. Calves given the IgM fraction and colostral whey orally had prolonged survival times ($p < 0.02$) over the other three groups and the onset of diarrhoea was significantly delayed ($p < 0.007$). Since it was demonstrated that the colostral whey had not been absorbed from the small intestine, it is concluded that colostral whey in addition to providing systemic immunity, has a local protective function within the gastro-intestinal tract.

Introduction

IN EARLIER experiments (Logan & Penhale, 1971; Penhale, Logan & Stenhouse, 1971), it was observed that either colostral whey or immunoglobulin M (IgM) fractions prepared from whey or bovine serum, inhibited septicaemia and prolonged survival time when administered intraperitoneally prior to experimental infection of colostrum-deprived calves. However, in these calves there was a high incidence of enteric disease as manifested clinically by severe diarrhoea which appeared to be little influenced by the prophylactic measures used. In view of this, and since colostrum, when acquired naturally by ingestion, may afford protection against both forms of colibacillosis, i.e., septicaemia and enterotoxic diarrhoea, it was suggested that the immunity was of a complex nature involving two separate systems; (1) systemic—mediated largely by IgM, preventing septicaemia and (2) local—within the lumen of the small intestine, inhibiting enteric disease. Such local immunity has been recorded in relation to enteric diseases in other species (Davies, 1922; Burrows, Dupree & Moore, 1950a, 1950b; Hooper & Haelterman, 1966; Kohler, 1967) but has not been demonstrated to our knowledge in colibacillosis in calves.

Enteric disease in calves is frequently accompanied by septicaemia and in order to investigate the local protective activity of colostral whey, it was necessary to provide systemic protection and then to administer whey orally after it has ceased to be absorbed from the small intestine.

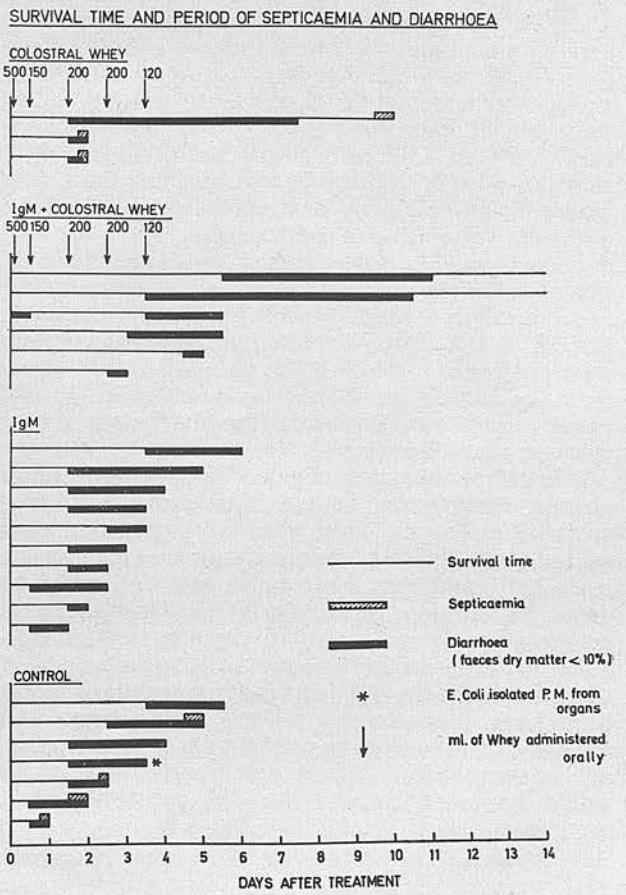

FIG. 1.—Serum immunoglobulin G levels in groups 3 and 4 before and after initial feeding of colostral whey.

Materials and Methods

Calves between three and seven days old were divided into four groups, as follows:—

1. Control calves which received no prophylactic treatment.
2. Calves which were given an IgM fraction intravenously (1·0 g. IgM/30 kg. bodyweight).
3. Calves which were given similar quantities of the IgM fraction and three hours later 500 ml. of pooled colostral whey orally and thereafter daily

doses of 150, 200, 200 and 120 ml. of whey. (Fig. 1).

4. Calves which were given only whey orally in doses similar to group 3.

The calves were then placed collectively in premises which had been contaminated by calves used in previous experiments. They were not experimentally infected, colibacillosis being allowed to develop spontaneously.

Calves

The calves were purchased from a dealer who had bought them at two markets in the West of Scotland. Using a zinc sulphate turbidity test to assess their serum immunoglobulin levels, they were screened and those with minimal readings, i.e., less than seven units, were used in the experiment. It was known from a preliminary experiment, and also from the results of Gay, Anderson, Fisher and McEwan (1965) that calves with readings of less than ten units were markedly or absolutely deficient in immunoglobulin and were susceptible to septicaemia.

On arrival, they were weighed, blood samples were taken for bacteriological and serological examination, and thereafter, twice daily throughout the experiment. Similarly, temperature, pulse and respiratory rates were measured. A daily faeces sample was taken from the rectum and dried to constant weight, a calf being considered diarrhoeic if the dry matter content was less than 10 per cent.

The calves were then divided into their respective groups, each group with a similar range of zinc sulphate readings. They were fed pasteurised milk according to appetite, up to a maximum of four pints twice daily and were kept under observation for 14 days. In addition, the serum IgG levels of calves in groups 3 and 4 were quantitatively measured immediately before oral administration of the first doses of whey and again the following morning. From Table I, it can be seen that the serum levels of IgG, before and after whey had been given were similar, indicating that no further absorption of immunoglobulin which would have interferred with the experiment had occurred.

TABLE I
SERUM IMMUNOGLOBULIN G LEVELS IN GROUPS 3 AND 4 BEFORE AND AFTER INITIAL FEEDING OF COLOSTRAL WHEY

Group	Calf number	IgG serum level mg. per ml.		
		On arrival	Before feeding whey	12 hrs. post feeding of whey
3 (IgM+Colostral whey)	162	0·19	0·41	0·30
	166	2·40	2·40	2·40
	167	0·24	0·51	0·41
	175	0·50	0·86	0·70
	180	0·80	0·64	0·71
	174	2·10	1·45	2·00
4 (Colostral whey)	163	0·26	0·33	0·20
	165	0·19	0·18	0·10
	181	0·17	0·21	0·20

Preparation of Colostral Whey

Colostrum taken at the first post-partum milking was collected from various farms. After pooling, fat was removed by centrifugation and whey was prepared by the addition of rennet. (Logan & Penhale, 1971).

Preparation of Immunoglobulin M Fraction

An IgM rich fraction was prepared by the standard method described by (Penhale, Logan & Stenhouse, 1971) but by cooling the blood to 4°C immediately after collection, the toxicity and consequent shock experienced using previous samples was minimal and the fraction was administered intravenously with no deleterious effects.

Quantitative Immunoglobulin Estimation

Immunoglobulin G was measured using the single radial diffusion technique (Penhale & Christie, 1969).

Zinc Turbidity Test

This was carried out using the method of McEwan, Fisher, Selman and Penhale, (1970) but the turbidity readings were made with an EEL* spectrophotometer. (McBeath, Penhale & Logan, 1971).

Bacteriology

Twice-daily, blood samples and swabs taken from various tissues of calves which died were examined bacteriologically for the presence of organisms. Isolated *E. coli* were tested by slide agglutination against standard antisera to serotype 078K80(B), (Logan & Penhale, 1971).

Results

Two calves were found on arrival to be septicaemic and were excluded from the experiment. The observations on the remaining calves are summarised in Fig. 1.

Of the 26 calves used in the experiment, only two which were given both IgM and colostral whey survived the observation period. Diarrhoea developed quickly, particularly in the control calves and also those given only IgM fraction, the first calf dying within 24 hours of being placed in the infected premises. Of the calves which were given IgM fraction intravenously, none became septicaemic, whereas of the other 10 calves, eight became septicaemic. *E. coli* serotype 078K80(B) which had been used extensively in these premises in earlier experiments was isolated from only one of the septicaemic calves.

1. *Control calves*

All seven calves in this group died, the mean survival time being 3·3 days (1 to 5·5 days). The calves began to scour shortly after being placed in the premises (Fig. 1) and once diarrhoea was established there was a rapid deterioration in condition. Terminally there was marked dehydration with haemoconcentration, the P.V.C. increasing by between 20 and 50 per cent. Most calves showed intermittent pyrexia, there being a rise in temperature of 2-3°F.

E. coli were isolated from the peripheral blood of

* Evans Electroselenium Ltd.

four calves, and on *post-mortem* examination, from these calves and a further calf, *E. coli* were cultured from the tissues. In only one case was the *E. coli* isolate identified as being serotype 078K80(B).

2. *Calves given IgM fraction intravenously*
Ten calves were each given IgM intravenously. All calves died; the mean survival time being 3·4 days (1·5 to 6 days). Clinically, these calves were similar to the control group, but *E. coli* was neither isolated *ante-mortem* from the peripheral blood nor at *post-mortem* examination, from the organs. However, large numbers of mucoid *E. coli* were regularly found in the upper small intestine and in some mesenteric lymph nodes.

3. *Calves given IgM fraction intravenously and colostral whey orally*
Of the six calves in this group, two were still surviving at the end of the experiment, and the mean survival time was 7·8 days (3 to 14+ days). In contrast to previous groups, the calves were bright, hungry and fed well for the first two-and-a-half days and of those which died, the first began to scour after 60 hours, the others following at intervals (Fig. 1). Again dehydration and haemoconcentration were observed. The two calves which survived began to scour after three-and-a-half and five-and-a-half days respectively, and although there was prolonged diarrhoea, these calves showed no evidence of dehydration, and throughout remained bright and hungry. Two of those which died began to scour whilst still receiving whey and subsequent daily doses did not alleviate the diarrhoea.

During the experiment, organisms were never isolated from the peripheral blood nor at *post-mortem* examination from the tissues, but mucoid strains of *E. coli* were again cultured from the proximal small intestine. Several of these mucoid strains of *E. coli* isolated from the small intestine and mesentive lymph nodes of the non-septicaemic diarrhoeic calves in groups 2 and 3 have been shown to belong to the known enteropathogenic strains "B42", "B85", "B117", and "B111" as originally identified by Smith and Halls (1967).

4. *Calves given whey orally*
Three calves were fed whey orally in doses similar to that of group 3 but without the IgM fraction parenterally. Within 24 hours, two of the calves showed pain and stiffness of the stifle joints. They became prostrate and died within two days, diarrhoea only being seen terminally. The third calf had diarrhoea for a prolonged period without dehydration but on the eighth day became dull, then recumbent and died on the tenth day.

All three calves had terminal septicaemia, *E. coli* being isolated from peripheral blood and at *post-mortem* examination from the tissues.

The results were statistically analysed by randomisation test. The survival times, and intervals before the onset of diarrhoea in groups 2 and 4, i.e., calves which received only IgM intravenously and whey orally respectively, were not significantly different to those of the control calves, group 1. In the calves of group 3 which received both IgM fraction and whey, the survival time was significantly prolonged ($p<0·02$) and the onset of diarrhoea was delayed ($p<0·007$). The administration of IgM fraction to groups 2 and 3 reduced the incidence of septicaemia ($p<0·005$), of the 16 calves in these groups none developed septicaemia, in contrast, of the 10 calves which were not given the IgM fraction, eight subsequently developed septicaemia.

Discussion

As 5/7 of the control calves died of colisepticaemia and the remaining two with diarrhoea, it can be assumed that all the calves were exposed to, and susceptible to, colibacillosis and that any significant difference in the inter group results could reasonably be attributed to the prophylactic treatment given.

In view of the clinical signs of scour, dehydration and haemoconcentration, which accompanied septicaemia in the majority of calves in the control group, it is likely that these calves, apart from the one which died within 24 hours, suffered from a septicaemic/enteric disease complex. It was interesting to note that although three weeks previously the premises had housed calves which had been experimentally infected with *E. coli* serotype 078K80(B), this serotype was found on only one occasion and this would suggest that the calves carried with them the other septicaemic strains isolated. This was supported by the finding that two calves were septicaemic on arrival.

The IgM fraction inhibited septicaemia but as previously observed (Penhale, Logan & Stenhouse, 1971), it failed to influence to any marked extent the enteric syndrome and it would appear that systemic antibodies play little part in this localised condition.

It is believed that the diarrhoea seen in these calves was associated with the presence in the small intestine of large numbers of mucoid *E. coli* which were demonstrated as being enteropathogenic strains.

In the group given both IgM and colostral whey orally, again septicaemia was prevented and in addition, the onset of scour was significantly delayed. The assumption that colostral whey would not be absorbed because of the age of the calves was subsequently confirmed by examination of sera for immunoglobulin. It is therefore concluded that the colostral whey immunoglobulin must have acted locally within the gastro-intestinal tract to inhibit enteric disease. This is in accord with other studies demonstrating that immunoglobulins can provide a local protective action within the gastro-intestinal tract (Davies, 1922; Burrows, *et al.*, 1947; 1948; 1950a; 1950b; Hooper & Haelterman, 1966; Kohler, 1967). Recent studies in other species (Tomasi, Tan, Solomon & Prendergast, 1965; South, Cooper, Wollheim, Hong & Good, 1966; Berger, Ainbender, Hodes, Zepp & Hevizy, 1967; Tourville, Adler, Bienenstock & Tomasi, 1969; Porter, Noakes & Allen, 1970) indicate that IgA is the principal immunoglobulin providing protection at the paramucosal surface and it is probable that in the calf colostral IgA (Mach, Pahud & Isliker, 1969) has a similar function. However, in the neonatal calf, protection is passive and other colostral immuno-

globulins, in addition to IgA, may contribute to this immunity.

The death from colisepticaemia of the calves which were only given whey orally further supports the conclusion that the colostral immunoglobulin administered was not absorbed from the intestine. Because of the short survival time of two of these calves, it was not possible to assess the influence of the whey on diarrhoea in this group.

In conclusion, these results indicate that two separate immunological systems are required to protect the neonatal calf against colibacillosis, one providing systemic protection the other local and for survival in a contaminated environment both must be present in adequate proportions.

Acknowledgments.—The authors are grateful to Messrs. A. Stenhouse D. J. Ormrod and R. J. McKenna for their capable technical help and to Mrs. J. Hurson, for the statistical analysis. We also thank Dr. E. W. Fisher for obtaining a supply of calves, Mr. D. G. McBeath for carrying out the zinc sulphate turbidity tests and Dr. W. J. Sojka who typed the *E. coli* strains from the small intestine.

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Résumé

Des veaux commerciaux de une semaine, souffrant d'hypogammaglobulinémie, ont été divisé en quatre groupes: (1) les sujets de contrôle, (2) les sujets qui reçurent une fraction riche en IgM par voie intraveineuse, (3) ceux qui reçurent une fraction de IgM par voie intraveineuse et buccale avec du petit-lait de colostrum et (4) ceux qui reçurent seulement du petit-lait de colostrum par voie buccale. Tous ces sujets furent ensuite placés dans des locaux antérieurement contaminés par des veaux porteurs de colibacilles.

Tous les sujets de contrôle moururent, cinq de colisepticémie, deux de diarrhée grave. La fraction IgM administrée seule, a dans tous les cas enrayé la septicémie mais pas la maladie entérique. Les sujets ayant reçu la fraction IgM et le petit-lait de colostrum eurent des temps de survie prolongés ($p < 0{,}02$) par rapport aux trois autres groupes et le début de la diarrhée était retardé d'une manière significative ($p < 0{,}007$). Après démonstration que le petit-lait de colostrum n'avait pas été absorbé à partir de l'intestin grèle, on en a conclu que le colostrum en plus de l'immunité systématique avait une fonction protectrice dans la voie gastro-intestinale.

Zusammenfassung

Zugekaufte hypogammaglobulinämische Kälber in der ersten Lebenswoche wurden in vier Gruppen eingeteilt: (1) Kontrollkälber, (2) Kälber, die intravenös eine IgM-reiche Fraktion erhielten, (3) Kälber, die intravenös eine IgM-Fraktion und peroral Kolostrum erhielten und (4) Kälber, die lediglich peroral Kolostrum erhielten. Die Tiere wurden in Räumen aufgestallt, die zuvor durch Kälber mit Kolibazillose kontaminiert worden waren.

Alle Kontrollkälber starben, u.zw. fünf an Kolisepsis und zwei an schwerem Durchfall. Die allein verabfolgte IgM-Fraktion konnte in sämtlichen Fällen die Sepsis verhüten, jedoch nicht die Darmerkrankung. Kälber, die die IgM-Fraktion sowie peroral Kolostrum erhielten, überlebten länger ($P < 0{,}02$) als die drei anderen Gruppen und der Durchfall setzte bedeutend später ein ($P < 0{,}007$). Aus der Tatsache, dass das Kolostrum nicht aus dem Dünndarm absorbiert wurde, wird geschlossen, dass Kolostrum nicht nur die Immunität eines bestimmten Körpersystems bewirkt sondern auch eine lokale Schutzfunktion innerhalb des Magen- und Darmkanals ausübt.

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Studies on the Immunity of the Calf to Colibacillosis

IV. The Prevention of Experimental Colisepticaemia by the Intravenous Administration of a Bovine Serum IgM-Rich Fraction

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SUMMARY.—The prophylactic effect of a double dose of a pooled bovine serum IgM rich fraction was investigated in two groups of experimentally challenged colostrum deprived calves, one group being kept during late summer, the other in mid-winter. It was found that the intravenous administration of two doses of the IgM fraction at a four day interval inhibited septicaemia in all calves but failed to prevent death from enteric disease in a proportion of the calves (2/12).

Introduction

IN EARLIER STUDIES (Logan & Penhale, 1971) it was found that colostral immunoglobulin of the IgM class prolonged survival time and delayed the onset of septicaemia when given intraperitoneally to neonatal colostrum-deprived calves prior to experimental challenge by a highly virulent strain of *E. coli*. Subsequently (Penhale, Logan & Stenhouse, 1971) it was demonstrated that a serum IgM preparation had similar activity and in some cases prevented colisepticaemia when given in doses in excess of 1 gm. IgM/30 kg. bodyweight. However, it was not possible to establish a precise protective dose against septicaemia. This inconsistency was thought to be due to a combination of factors:—

1. Irregularities in the absorption of immunglobulin may have occurred when the fraction was given intraperitoneally.
2. Variation in the severity of diarrhoea could affect the half life of immunoglobulin (MacDougall & Mulligan, 1969).
3. It is likely that IgM is rapidly catabolised and thus, at the dosage levels which were found practicable, may have resulted in sub protective levels in the circulation within a few days of administration.
4. Toxic factors known to be present in earlier IgM preparations prevented administration of doses in excess of 2 gms. IgM and therefore, paradoxically, may have reduced the calves resistance to the *E. coli* challenge particularly at the higher dosage levels.

In order to investigate these possibilities, attention was given to the preparation of a sterile non-toxic IgM fraction which could be given intravenously.

Materials and Methods

Preparation of the Serum IgM Fraction

In a preliminary experiment, it was observed that, when a sterile IgM fraction was prepared from blood obtained aseptically from living cattle, it could be administered by the intravenous route to neonatal calves without causing either the clinical signs or haemodynamic changes characteristic of shock. Accordingly, bulk quantities of the IgM fraction were prepared from abbatoir blood by the method previously described (Penhale, Logan & Stenhouse, 1971) but with the modification that all pooled blood was checked for sterility and only samples with minimal bacterial contamination (20 colonies/ml.) were used. Similar checks were also carried out at strategic points during the fractionation process.

Calves

Newborn colostrum-deprived Ayrshire bull calves, obtained from a closed herd were managed as previously described (Logan & Penhale, 1971). Six calves were given a single intravenous injection of the IgM rich serum fraction, containing a standard dose of 1 gm. IgM/30 kg. bodyweight and two hours later, they were infected orally with the experimental *E. coli* serotype. Twelve calves were similarly treated, but in addition were given a further dose of the preparation intravenously on the fourth day post-challenge because, in earlier experiments (Logan & Penhale, 1971; Penhale, Logan & Stenhouse, 1971), it was found that the onset of septicaemia was delayed approximately four days when a single dose was administered intraperitoneally. To avoid the possibility of favourable seasonal conditions seven calves were treated during the late summer and early autumn (August-October) and five were treated during mid-winter (December-February). During the course of the experiment the virulence of the *E. coli* organism was checked by regularly challenging untreated control calves. Calves were kept under observation over a period of 14 days during which time blood samples were taken twice daily for bacteriological and serological examination.

Escherichia coli

As in earlier experiments (Logan & Penhale, 1971; Penhale, Logan & Stenhouse, 1971) calves were infected orally with 1 ml./10 kg. bodyweight of a six hour glucose broth culture of a highly virulent strain of *E. coli* 078K80(B).

Bacteriology

Daily blood samples and swabs taken from various organs of calves which died, were examined bacteriologically and isolated colonies of *E. coli* were tested by slide agglutination with standard antisera against 078K80(B) as described previously (Penhale 1965, Logan & Penhale, 1971). Preliminary observations were also made on the intestinal flora of those calves which died; particular attention being paid to the isolation of mucoid strains of *E. coli* from the proximal small intestine.

Quantitative Immunoglobulin Determination
The daily serum levels of immunoglobulins were measured quantitatively by the single radial diffusion technique of Mancini, Carbonora and Heremans (1965) as adapted by Penhale and Christie (1969).

Indirect Haemagglutination and Antiglobulin Tests
An 0 antigen extract of *E. coli* serotype 078K80(B) was prepared and treated for use in haemagglutination and antiglobulin tests according to the method described by Penhale (1965).

Results

The serum IgM fraction, when prepared from sterile blood, was found generally to be well tolerated when administered by the intravenous route. In some cases following injection no observable reaction occurred but, more frequently, slight depression and hyperpnea were noted immediately post-injection, and occasionally this was followed by muscular weakness and recumbency. In all cases where a reaction was seen this was of short duration and calves invariably recovered within five minutes. Up to the present, 47 calves have been given this sterile preparation intravenously and no deaths have occurred.

Prophylactic Studies
The prophylactic effects of this preparation in the experimentally infected calves are summarised in Figs. 1 and 2. The six calves which received one

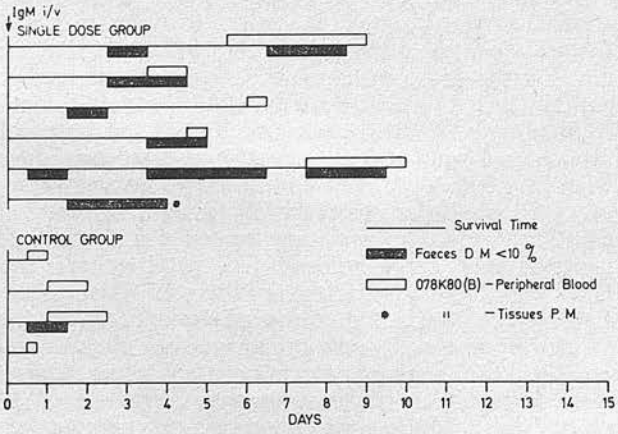

FIG. 1.—Survival times and periods of diarrhoea and septicaemia in control calves and calves receiving one dose of serum IgM fraction.

dose of IgM fraction, died of septicaemia. The experimental serotype was recovered from the peripheral blood in five calves and from the organs *post-mortem* in every case. The mean survival time (six days post-challenge) and delay in onset of septicaemia (five days) was comparable to that seen in calves receiving colostral whey, colostral IgM or serum IgM intraperitoneally (Logan & Penhale 1971, Penhale, Logan & Stenhouse, 1971).

In contrast, none of the calves receiving two doses of the serum IgM fraction became bacteraemic, except on one occasion when 20 cols/ml. were isolated from the blood of one calf on the ninth day (Fig. 2). This calf, at that time, appeared to be clinically normal and it may be significant that it was penned adjacent to one which had been infected 24 hours previously. Of the 12 calves, ten survived, while one in each group died of enteric disease. Bacterial examination of these two calves *post-mortem* failed to demonstrate the presence of *E. coli* in the tissues, but mucoid strains of *E. coli* were isolated from the small intestine and mesenteric lymph nodes. As in previous experiments (Logan & Penhale, 1971, Penhale, Logan & Stenhouse, 1971) diarrhoea was seen apart from one exception in all calves.

The untreated control calves (Fig. 1) became bacteraemic within 24 hours of challenge and died of septicaemia within 60 hours. This pattern conformed with previous experience using this serotype in colostrum-deprived calves (Fey & Margadant 1963; Penhale 1965; Smith & Halls 1968; Logan & Penhale 1971; Penhale, Logan & Stenhouse, 1971).

Serology
The pre-injection serum samples contained neither antiglobulin nor haemagglutination activity against the 0 antigen of challenge serotype (Figs. 3 and 4). After administration of the IgM serum fraction, all calves showed an initial rise in both antiglobulin and haemagglutination titres, following which there was rapid decline to negligible levels at the fourth day post challenge. In the single dose group this was followed by a rise on the fifth day (Fig. 3).

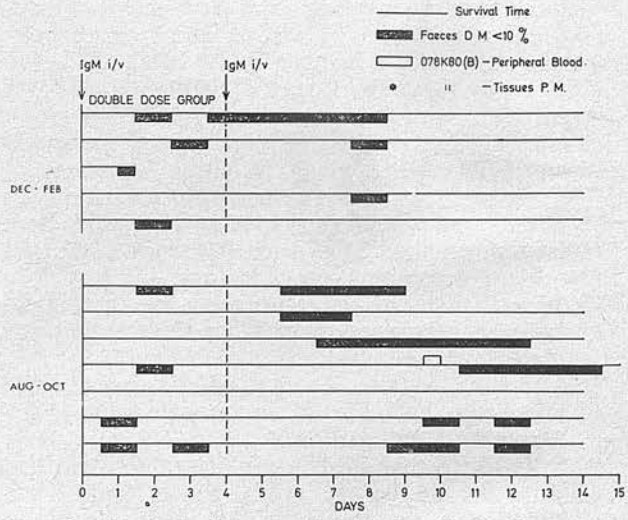

FIG. 2.—Survival times and periods of diarrhoea and septicaemia in calves receiving two doses of IgM fraction.

In the case of those receiving the double dose (Fig. 4), a second peak of antibody activity corresponded with the time of injection of the second dose, after which antiglobulin and haemagglutinating titres again declined until the seventh day when levels once more began to rise slowly.

The daily serum IgM levels followed a similar pattern to the haemagglutination titres. The IgG serum levels in both groups of calves increased gradually from birth, but throughout the observation period remained low relative to adult or colostrum-fed calf levels (Penhale & Christie, 1969; Klaus, Bennett & Jones, 1969).

The very wide standard deviation seen in both IgG and IgM levels was due to the fact that, while the majority of the calves had no immunoglobulin prior to injection of the IgM fraction, a few had measurable amounts of foetal immunoglobulin at birth, particularly of IgG. The levels of immuno-

globulin in these calves remained consistently higher throughout the observation period.

Discussion

These results indicate that a non-toxic serum IgM fraction, at the dosage level employed, can consistently protect colostrum deprived calves against experimental colisepticaemia if given intravenously in two doses. The result did not appear to be influenced by any seasonal variation, one calf in each group, kept at different periods, dying of enteric disease. During this experiment, while the calves suffered from diarrhoea, it did not appear to be quite so severe as in earlier experiments (Logan & Penhale, 1971; Penhale, Logan & Stenhouse, 1971) and it may be that the enteropathogenic *E. coli*, which spontaneously infected calves was not as virulent as in earlier studies. On the other hand, the earlier IgM preparations were known to be toxic and in consequence may have depressed the resistance of the calves or perhaps even actively precipitated the diarrhoea. In the latter context, it has been stated (Wray & Thomlinson, 1969) that *E. coli* endotoxin caused enteritis when given parenterally to calves. As the survival times and delay in onset of septicaemia in the single dose group were comparable to those of calves receiving either colostral whey or IgM fractions intraperitoneally (Logan & Penhale, 1971; Penhale, Logan & Stenhouse, 1971) it is likely that irregular absorption from the peritoneum was not the primary factor in the failure to prevent septicaemia in earlier experiments. The rapid post injection fall in antibody titres accompanied by a drop in serum IgM levels, however, indicate that the half life of bovine IgM like that of other species (Uhr & Finkelstein, 1963; Frommel, Perey & Good, 1970; Porter & Hill, 1970) is quite short—probably less than five days. It is probable, therefore, that the short half life of this immunoglobulin may account for the failure to prevent septicaemia when one dose was administered. In this connection, if it is assumed that the half life of IgM is four days, it can be seen (Fig. 5) that two doses, given at an interval of four days, provide substantially higher circulating levels during the period when the onset of septicaemia occurred in the single dose calves than if the same quantity of IgM is given as a single dose on the first day.

Plasma IgG levels in all pre-treated calves showed a tendency to rise steadily from birth. Nevertheless, levels were still relatively low as compared to adult levels at the end of the experimental period. (Penhale & Christie, 1970). Plasma IgM levels showed a similar increase from birth superimposed upon the sharp increases immediately following the administration of the IgM fraction. In contrast to IgG, IgM levels in calves which survived were approaching those of the adult at the end of the experimental period. In the single dose group an abrupt rise in plasma IgM levels accompanied the onset of septicaemia suggesting that this represented a specific response to the antigens present in the challenge organism.

Fluctuations in the haemagglutination titres to the 0 antigen of the challenge strain of *E. coli* closely paralleled those of the plasma IgM indicating that these antibodies were mainly present in this particular class of immunoglobulin.

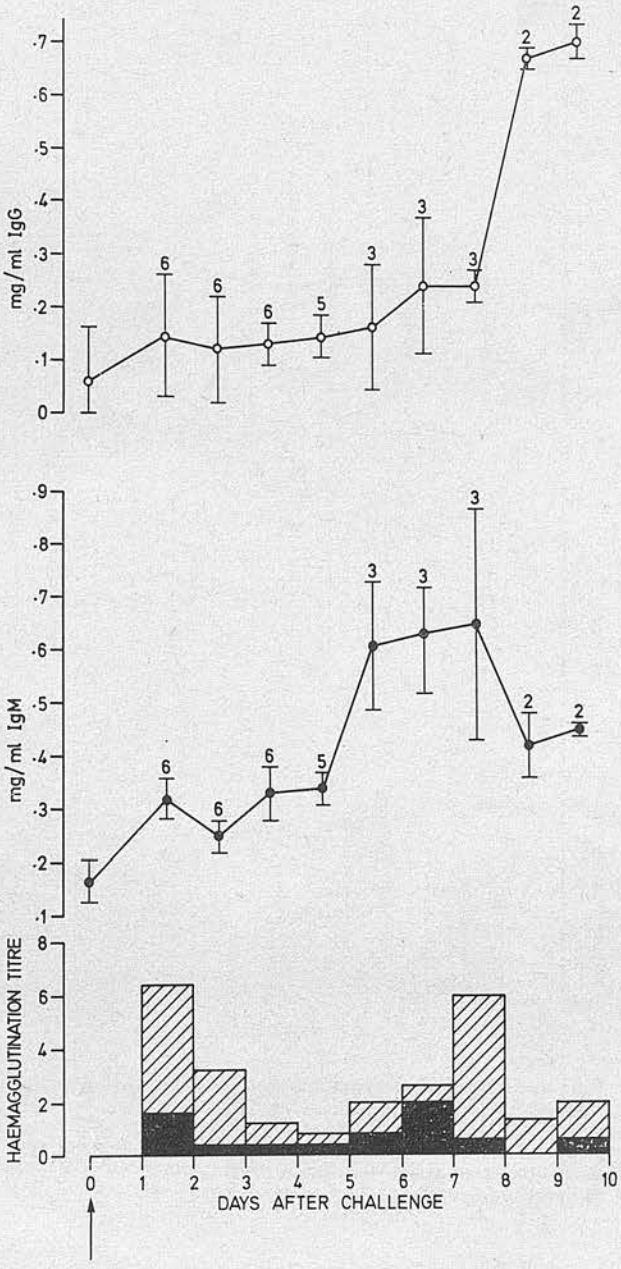

FIG. 3.—Plasma haemagglutination and antiglobulin titres, IgG and IgM levels with standard deviations, in calves receiving one dose of serum IgM fraction. Arrow indicates time of injection of IgM fraction; haemagglutination titre represents the arithmetical mean of the daily indirect haemagglutination titres (black) and antiglobulin titres (shaded) figures. Above S.D. are the numbers of calves sampled.

The evidence obtained of active antibody production by both quantitative immunoglobulin determinations and the measurement of antibody levels by antiglobulin haemagglutination tests is consistent with the findings of others (Brown, 1956; Fennestad & Borg-Petersen, 1957; & Penhale, 1965) that the calf is immunologically competent at birth and it would appear that it has the capacity to produce immunoglobulins of both IgG & IgM classes at this time. Moreover, relative to adult levels, IgM synthesis would seem to be more efficient at this stage than IgG.

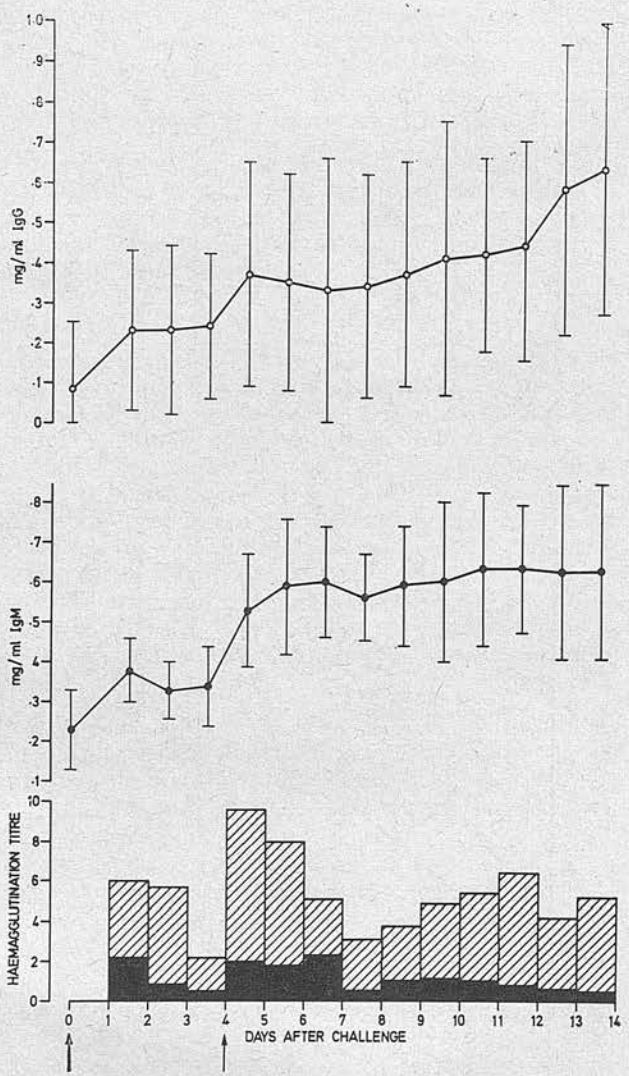

FIG. 4.—Plasma haemagglutination and antiglobulin titres, IgG and IgM levels with standard deviations, in calves receiving two doses of serum IgM fraction. Arrows indicate times of injection of IgM fraction; haemagglutination titre represents the arithmetical mean of the daily indirect haemagglutination titres (black) and antiglobulin titres (shaded).

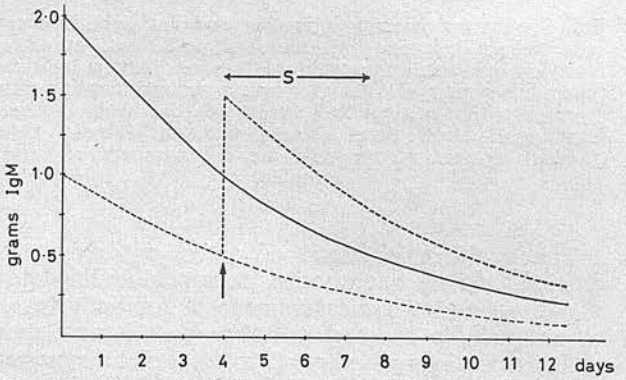

FIG. 5.—Theoretical decay curve of IgM having a half life of four days ← S → represents period when onset of septicaemia was observed in single dose calves. Arrow indicates time of second injection.

In conclusion, the present experiments clearly show that colostrum-deprived calves can be protected against experimental challenge with a highly virulent invasive strain of *E. coli* by the administration of relatively small amounts of immunoglobulin during the early neonatal period and also provide further evidence that the immunoglobulins of the IgM class are the most significant in this respect. In this connection, it is of interest to note that those calves which survived as a consequence of the administration of the serum IgM fraction had plasma IgG levels approximately similar to those of market calves which invariably died of septicaemia (Penhale & Christie, McEwan, Fisher & Selman, 1970).

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Résumé

On a étudié l'efficacité prophylactique d'une double dose de la fraction riche d'un sérum bovin IgM complexe sur deux groupes de veaux auxquels on avait fait auparavant l'ablation du colostrum; ces expériences ont été pratiquées à la fin de l'été pour le premier groupe, et au milieu de l'hiver pour le second groupe. On s'est aperçu qu'on pouvait enrayer tous les cas de septicémie par l'administration par intra-veineuses de deux doses de IgM à quatre jours d'intervalle, mais on n'a pas pu empêcher la mort par entéralgie de deux douzième des animaux.

Zusammenfassung

Die prophylaktische Wirkung einer doppelten Dosis Sammelrinderserum mit angereicherter IgM-Fraktion wurde an zwei Gruppen von experimentell mit Kolibazillen infizierten Kälbern unter Kolostrumentzug geprüft. Eine Gruppe wurde im Spätsommer gehalten, die andere in der Mitte des Winters. Es wurde festgestellt, dass die intravenöse Verabfolgung von 2 Dosen der IgM-Fraktion in Zeitabständen von vier Tagen bei sämtlichen Kälbern Septikämie verhütete, dass sie jedoch nicht vermochte, den Tod eines Teiles der Kälber (2/12) infolge Darmerkrankung abzuwenden.

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